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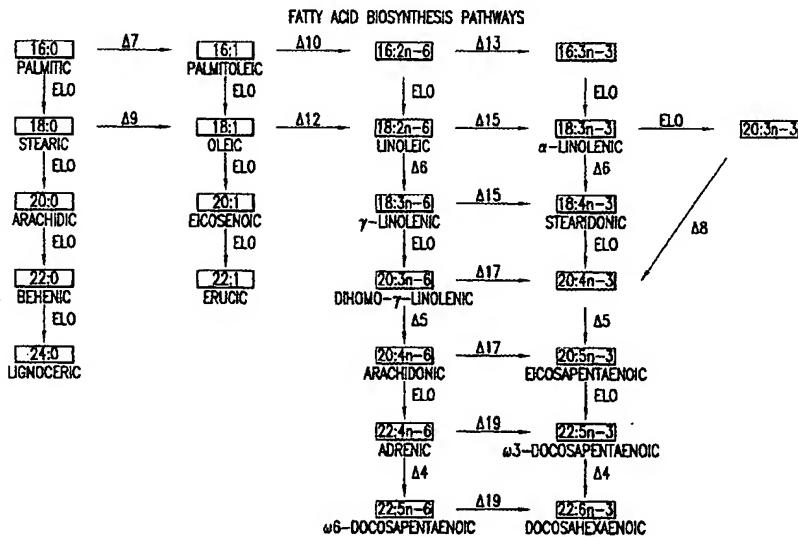
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(54) Title: ELONGASE GENES AND USES THEREOF



(57) Abstract: The subject invention relates to the identification of several genes involved in the elongation of polyunsaturated acids (i.e., "elongases") and to uses thereof. At least two of these genes are also involved in the elongation of monounsaturated fatty acids. In particular, elongase is utilized in the conversion of gamma linolenic acid (GLA) to dihomogamma linolenic acid (DGLA) and in the conversion of DGLA or 20:4n-3 to eicosapentaenoic acid (EPA). DGLA may be utilized in the production of polyunsaturated fatty acids, such as arachidonic acid (AA), docosahexaenoic acid (DHA), EPA, adrenic acid, w6-docosapentaenoic acid or w3-docosapentaenoic acid which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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**ELONGASE GENES AND USES THEREOF**

The subject application is a Continuation-In-Part of pending U.S. patent application Serial No. 09/624,670 filed on 5 July 24, 2000, which is a Continuation-In-Part of pending U.S. patent application Serial No. 09/379,095 filed on August 23, 1999, which is a Continuation-In-Part of pending U.S. patent application Serial No. 09/145,828 filed on September 2, 1998, all of which are herein incorporated in their entirety by 10 reference.

**BACKGROUND OF THE INVENTION****Technical Field**

The subject invention relates to the identification of 15 several genes involved in the elongation of long-chain polyunsaturated fatty acids (i.e., "elongases") and to uses thereof. In particular, the elongase enzyme is utilized in the conversion of one fatty acid to another. For example, elongase catalyzes the conversion of gamma linolenic acid (GLA) to 20 dihomo- $\gamma$ -linolenic acid (DGLA, 20:3n-6) and the conversion of stearidonic acid (STA, 18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Elongase also catalyzes the conversion of arachidonic acid (AA, 20:4n-6) to adrenic acid (ADA, 22:4n-6), the conversion of eicosapentaenoic acid (EPA, 20:5n-3) to  $\omega$ 3-docosapentaenoic acid (22:5n-3), and the conversion of  $\alpha$ -linolenic acid (ALA, 18:3n-3) to 20:3n-3. DGLA, for example, may be utilized in the production of other polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) which may be added to pharmaceutical compositions, nutritional compositions, animal 25 feeds, as well as other products such as cosmetics.

Background Information

The elongases which have been identified in the past differ in terms of the substrates upon which they act. Furthermore, 5 they are present in both animals and plants. Those found in mammals have the ability to act on saturated, monounsaturated and polyunsaturated fatty acids. In contrast, those found in plants are specific for saturated or monounsaturated fatty acids. Thus, in order to generate polyunsaturated fatty acids 10 in plants, there is a need for a PUFA-specific elongase.

In both plants and animals, the elongation process is believed to be the result of a four-step mechanism (Lassner et al., The Plant Cell 8:281-292 (1996)). CoA is the acyl carrier. Step one involves condensation of malonyl-CoA with a long-chain 15 acyl-CoA to yield carbon dioxide and a  $\beta$ -ketoacyl-CoA in which the acyl moiety has been elongated by two carbon atoms. Subsequent reactions include reduction to  $\beta$ -hydroxyacyl-CoA; dehydration to an enoyl-CoA, and a second reduction to yield the elongated acyl-CoA. The initial condensation reaction is not 20 only the substrate-specific step but also the rate-limiting step.

As noted previously, elongases, more specifically, those which utilize PUFAs as substrates, are critical in the production of long-chain polyunsaturated fatty acids which have 25 many important functions. For example, PUFAs are important components of the plasma membrane of a cell where they are found in the form of phospholipids. They also serve as precursors to mammalian prostacyclins, eicosanoids, leukotrienes and prostaglandins. Additionally, PUFAs are necessary for the 30 proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological

significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, efficiently.

A number of enzymes are involved in PUFA biosynthesis  
5 including elongases (elo) (see Figure 1). For example, linoleic acid (LA, 18:2- $\Delta$ 9,12 or 18:2n-6) is produced from oleic acid (OA, 18:1- $\Delta$ 9 or 18:1n-9) by a  $\Delta$ 12 desaturase. GLA (18:3- $\Delta$ 6,9,12) is produced from linoleic acid by a  $\Delta$ 6-desaturase. AA (20:4- $\Delta$ 5,8,11,14) is produced from dihomoo- $\gamma$ -linolenic acid (DGLA, 10 20:3- $\Delta$ 8,11,14) by a  $\Delta$ 5-desaturase. As noted above, DGLA is produced from GLA by an elongase.

It must be noted that animals cannot desaturate beyond the  $\Delta$ 9 position and therefore cannot convert oleic acid into linoleic acid. Likewise,  $\alpha$ -linolenic acid (ALA, 18:3- $\Delta$ 9,12,15 or 18:3n-3) cannot be synthesized by mammals, since they lack  $\Delta$ 15 desaturase activity. However,  $\alpha$ -linolenic acid can be converted to stearidonic acid (STA, 18:4- $\Delta$ 6,9,12,15) by a  $\Delta$ 6-desaturase (see PCT publication WO 96/13591; see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4- $\Delta$ 8,11,14,17 or 20:4n-3) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4- $\Delta$ 8,11,14,17) can then be converted to eicosapentaenoic acid (EPA, 20:5- $\Delta$ 5,8,11,14,17) by a  $\Delta$ 5-desaturase. Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbons 12 (see PCT publication 25 WO 94/11516 and U.S. Patent No. 5,443,974) and 15 (see PCT publication WO 93/11245). The major polyunsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or  $\alpha$ -linolenic acid. In view of the inability of mammals to produce these essential 30 long chain fatty acids, it is of significant interest to isolate

genes involved in PUFA biosynthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant or animal system which can be altered to provide production of commercial quantities of one or more 5 PUFAs. Consequently, there is a definite need for the elongase enzyme, the gene encoding the enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFA beyond those naturally present as well as those enriched in novel PUFAs. Such oils can 10 only be made by isolation and expression of the elongase gene.

One of the most important long chain PUFAs, noted above, is arachidonic acid (AA). AA is found in filamentous fungi and can also be purified from mammalian tissues including the liver and the adrenal glands. As noted above, AA production from DGLA is 15 catalyzed by a  $\Delta$ 5-desaturase, and DGLA production from  $\gamma$ -linolenic acid (GLA) is catalyzed by an elongase. However, until the present invention, no elongase had been identified which was active on substrate fatty acids in the pathways for the production of long chain PUFAs and, in particular, AA, 20 eicosapentaenoic acid (EPA), adrenic acid, docosahexaenoic acid (DHA, 22:6n-3),  $\omega$ 3-docosapentaenoic acid (22:5n-3) or  $\omega$ 6-docosapentaenoic acid (22:5n-6).

Two genes appeared to be of interest in the present search for the elongase gene. In particular, the jojoba  $\beta$ -ketoacyl-25 coenzyme A synthase (KCS), or jojoba KCS (GenBank Accession # U37088), catalyzes the initial reaction of the fatty acyl-CoA elongation pathway (i.e., the condensation of malonyl-CoA with long-chain acyl-CoA (Lassner et al., The Plant Cell 8:281-292 (1996)). Jojoba KCS substrate preference is 18:0, 20:0, 20:1, 30 18:1, 22:1, 22:0 and 16:0. Saccharomyces cerevisiae elongase

(ELO2) also catalyzes the conversion of long chain saturated and monounsaturated fatty acids, producing high levels of 22:0, 24:0, and also 18:0, 18:1, 20:0, 20:1, 22:0, 22:1, and 24:1 (Oh et al., The Journal of Biological Chemistry 272 (28):17376-17384 5 (1997); see also U.S. Patent No. 5,484,724 for a nucleotide sequence which includes the sequence of ELO2; see PCT publication WO 88/07577 for a discussion of the sequence of a glycosylation inhibiting factor which is described in Example V). The search for a long chain PUFA-specific elongase in 10 Mortierella alpina began based upon a review of the homologies shared between these two genes and by expression screening for PUFA-elongase activity.

#### SUMMARY OF THE INVENTION

15 The present invention relates to an isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence shown in SEQ ID NO:1 (Figure 6). This isolated sequence may be represented by SEQ ID NO:1. The sequence encodes a functionally active elongase which utilizes a 20 polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. In particular, the sequence may be derived from a fungus of the genus Mortierella and may specifically be isolated from Mortierella alpina.

25 The present invention also includes a purified protein encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids or monounsaturated fatty acids and has at least about 50% amino acid similarity to the amino acid sequence of the purified protein encoded by the above nucleotide sequence.

30 Additionally, the present invention encompasses a method of producing an elongase enzyme comprising the steps of: a)

isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 6); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and  
5 conditions sufficient for expression of the elongase enzyme.

The host cell may be a eukaryotic cell or a prokaryotic cell.

The prokaryotic cell may be, for example an E. coli cell, a cyanobacterial cell, or a B. subtilis cell. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant  
10 cell or a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp.,  
15 Penicillium spp., Neurospora spp., Trichoderma spp. or Pichia spp.. In particular, the fungal cell may be a yeast cell such as  
15 Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. or Pichia spp..

The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 6) operably linked to b) a promoter, as well as a host cell comprising this vector. The host may be a prokaryotic cell or a eukaryotic cell. Suitable examples of prokaryotic cells include E. coli, Cyanobacteria, and B. subtilis cells. Suitable examples of eukaryotic cells include a mammalian cell, an insect cell, a plant cell and a fungal cell. The fungal cell may be,  
20 for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.. In particular, the fungal cell may be, for example, a yeast cell such as, for example, Saccharomyces spp., in  
25 particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. and Pichia spp..

The present invention includes a plant cell, plant or plant tissue comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of  
5 a monounsaturated fatty acid and a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, dihomo- $\gamma$ -linolenic acid (DGLA), 20:4n-3, and adrenic acid (ADA). The invention also includes one or more plant oils or fatty acids expressed by the plant  
10 cell, plant or plant tissue. Additionally, the present invention encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

15 Furthermore, the present invention includes a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:1 (Figure 6). The present invention also includes a fluid (e.g., milk) produced by the transgenic,  
20 non-human wherein the fluid comprises a detectable level of at least one elongase or products thereof such as, for example, DGLA,  $\omega$ 6-docosapentaenoic acid, ADA and/or 20:4n-3 (see Figure 1).

25 Additionally, the present invention includes a method for producing a polyunsaturated fatty acid comprising the steps of:  
a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 6); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase  
30 enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a "substrate"

polyunsaturated fatty acid in order to convert the substrate to a "product" polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be selected from the group consisting of, for example,  $\gamma$ -linolenic acid (GLA), stearidonic acid (STA) and arachidonic acid (AA), and the product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to "another" polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, eicosapentaenoic acid (EPA),  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase, with respect to production of AA or EPA, and  $\Delta$ 4-desaturase, with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a "final" polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid (DHA), AA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

Also, the present invention includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-

described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be  
5 selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or ω6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid, ω6-docosapentaenoic acid or ω3-docosapentaenoic acid.  
10 The nutritional composition may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or an animal and may be administered enterally or parenterally. The nutritional composition may further comprise at least one macronutrient selected from the  
15 group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil. It may also comprise at  
20 least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.  
25 Additionally, the present invention encompasses a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid  
30 produced according to the above-described method, and the final

polyunsaturated fatty acid produced according to the above-described method and 2) a pharmaceutically acceptable carrier. The composition may be administered to a human or an animal. It may also further comprise at least one element selected from the  
5 group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound. It may be administered enterally, parenterally,  
10 topically, rectally, intramuscularly, subcutaneously, intradermally, or by any other appropriate means.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid  
15 produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3,  
20 and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

Moreover, the present invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid  
25 produced according to the above-described method.

Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the above nutritional composition 5 in an amount sufficient to effect prevention or treatment.

The present invention also includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:2 (Figure 22). This sequence may be represented by SEQ ID NO:2. The sequence 10 encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. This sequence may also be derived, for example, from a fungus of the genus Mortierella. In particular, it may be derived from M. alpina.

Additionally, the present invention includes a purified 15 protein encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

The present invention also includes a method of producing 20 an elongase enzyme as described above. The sequence inserted in the vector is represented by SEQ ID NO:2 (Figure 22). The host cell may be prokaryotic or eukaryotic. Suitable examples are described above.

The present invention also includes a vector comprising: a) 25 a nucleotide sequence as represented by SEQ ID NO:2 (Figure 22) operably linked to b) a promoter, as well as a host cell comprising this vector. Again, the host cell may be eukaryotic or prokaryotic. Suitable examples are described above.

The invention also includes a plant cell, plant or plant 30 tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a

polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. Additionally, the invention includes one or more plant oils or fatty acids expressed by the plant cell,  
5 plant or plant tissue.

Furthermore, the present invention also includes a transgenic plant comprising the above vector, wherein expression of the nucleotide sequence (SEQ ID NO:2) of the vector results in production of a polyunsaturated fatty acid in seeds of the  
10 transgenic plant.

The invention also includes a transgenic, non-human mammal whose genome comprises a DNA sequence (SEQ ID NO:2) encoding an elongase operably linked to a promoter. The invention also includes a fluid produced by this transgenic, non-human mammal  
15 wherein the fluid comprises a detectable level of at least one elongase or products thereof.

The present invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:2  
20 (Figure 22); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate  
25 polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA, or AA, the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or  $\omega$ 6-docosapentaenoic acid, respectively.  
30 The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to

convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-  
5 docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step  
10 of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, docosahexaenoic acid, AA,  $\omega$ 6-  
15 docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be selected from the group consisting of, for example, AA, EPA, and  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be selected from the group consisting of, for example, DHA, AA,  $\omega$ 6-  
30 docosapentaenoic acid, and  $\omega$ 3-docosapentaenoic acid.

The other attributes of the composition are the same as those described above with respect to administration, characterization, components, etc.

The present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method of noted above with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and 2) a pharmaceutically acceptable carrier. The characteristics of the above-described pharmaceutical composition (e.g., administration, components, etc.) also apply to this composition.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2, the another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described with respect to SEQ ID NO:2.

The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, the 5 another polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2, and the final polyunsaturated fatty acid produced according to the method described above with respect to SEQ ID NO:2.

Additionally, the present invention includes a method of 10 preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition described directly above in an amount sufficient to effect the prevention or treatment.

15 Furthermore, the present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence shown in SEQ ID NO:3 (Figure 43). This sequence may be that represented by SEQ ID NO:3. This sequence encodes a functionally active elongase 20 which utilizes a polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. The sequence is derived from a mammal such as, for example, a human.

The invention also includes a purified protein encoded by 25 this nucleotide sequence. Also, the invention includes a purified polypeptide which elongates polyunsaturated fatty acids or monounsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of this purified protein.

Additionally, the invention includes method of producing an 30 elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b)

constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing said vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The host cell  
5 may be the same as that described above with respect to the corresponding methods utilizing SEQ ID NO:1 or 2.

The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:3 (Figure 43) operably linked to b) a promoter, as well as a host cell  
10 comprising this vector. The host cell may be the same as that described above.

The invention also includes a plant cell, plant or plant tissue comprising the above-described vector comprising SEQ ID NO:3, wherein expression of the nucleotide sequence of the  
15 vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by said plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The invention also includes one or more  
20 plant oils or acids expressed by the plant cell, plant or plant tissue.

The invention also includes a transgenic plant comprising the vector comprising SEQ ID NO:3, wherein expression of the nucleotide sequence of the vector results in production of a  
25 polyunsaturated fatty acid in seeds of the transgenic plant.

Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a human DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence is represented by SEQ ID NO:3 (Figure 43). The  
30 invention also includes a fluid produced by said transgenic,

non-human mammal wherein said fluid comprises a detectable level of at least one elongase or products thereof.

The invention also encompasses a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating 5 the nucleotide sequence represented by SEQ ID NO:3 (Figure 43); b) constructing a vector comprising said nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the 10 expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA or AA, and the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, 15 or ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one desaturase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 and ADA, the another polyunsaturated fatty acid may be, for 20 example, AA, EPA, and  $\omega 6$ -docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta 5$ -desaturase with respect to production of AA or EPA and  $\Delta 4$ -desaturase with respect to production of  $\omega 6$ -docosapentaenoic acid. The method may further 25 comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated

fatty acid may be, for example, DHA, ADA,  $\omega 6$ -docosapentaenoic acid, and  $\omega 3$ -docosapentaenoic acid.

The nutritional composition comprising at least one polyunsaturated fatty acid which may be, for example, product 5 polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method 10 recited above in connection with SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be selected from the group consisting of AA, EPA, or  $\omega 6$ -docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, 15 DHA, ADA,  $\omega 6$ -docosapentaenoic acid, or  $\omega 3$ -docosapentaenoic acid. The other properties or characteristic of the nutritional composition (e.g., administration, components, etc.) as the same as those recited above with respect to the other nutritional compositions.

20 Moreover, the present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3, the 25 another polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:3 and 2) a pharmaceutically acceptable carrier. The other properties of 30 the composition (e.g., administration, additional components,

etc.) are the same as those recited above with respect to the other pharmaceutical compositions.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, the another polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

Also, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to the method recited above with respect to SEQ ID NO:3, said another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:3.

A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional composition recited above in connection with SEQ ID NO:3 in an amount sufficient to effect the prevention or treatment.

Additionally, the present invention includes an isolated nucleotide sequence corresponding to or complementary to at

least about 35% of the nucleotide sequence shown in SEQ ID NO:4 (Figure 46). The sequence may be represented by SEQ ID NO:4. It encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. The sequence may be 5 derived or isolated from a nematode of the genus Caenorhabditis and, in particular, may be isolated from C. elegans.

The present invention includes a purified protein encoded by the nucleotide sequence above. The invention also includes a purified polypeptide which elongates polyunsaturated fatty acids 10 and has at least about 30% amino acid similarity to the amino acid sequence of the purified protein.

Additionally, the present invention includes a method of producing an elongase enzyme comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:4 15 (Figure 46); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the elongase enzyme. The properties of the host cell are the same as those described 20 above in connection with SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

The present include also encompasses a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:4 (Figure 46) operably linked to b) a promoter, as well as a host cell 25 comprising this vector. The host cell has the same properties as those recited above in connection with the host cell recited above for SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

Moreover, the present invention includes a plant cell, plant or plant tissue comprising the above vector comprising SEQ 30 ID NO:4, wherein expression of said nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by

the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The invention also includes one or more plant oils or fatty acids expressed by this plant cell, plant or plant tissue.

5       The invention also includes transgenic plant comprising the above vector including the nucleotide sequence corresponding to SEQ ID NO:4, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

10      Additionally, the present invention includes a transgenic, non-human mammal whose genome comprises a C. elegans DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:4 (Figure 46). The invention also includes a fluid produced by the transgenic, 15 non-human mammal, wherein the fluid comprises a detectable level of at least one elongase or products thereof.

10      The invention also includes a method for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:4 (Figure 46);  
20 b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by the isolated nucleotide sequence; and d) exposing the expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, GLA, STA, or AA, and the product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA, respectively. The method may further comprise the step  
25 of exposing the expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated

fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA, the another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

The invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said the polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid. The other characteristics of the composition are the same as those recited for the nutritional compositions present above.

Additionally, the present invention includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of: the product polyunsaturated fatty acid produced according to 5 the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4 and 2) a 10 pharmaceutically acceptable carrier. The composition has the same properties (e.g., administration, added elements, etc.) as those described above with respect to the other pharmaceutical compositions.

The present invention also includes an animal feed 15 comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection 20 with SEQ ID NO:4, and the final polyunsaturated fatty acid produced according to the method described above in connection with SEQ ID NO:4. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3 or ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA or  $\omega$ 6- 25 docosapentaenoic acid. The polyunsaturated fatty acid may be, for example, DHA, ADA,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

Additionally, the present invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group 30 consisting of the product polyunsaturated fatty acid produced

according to the method recited above in connection with SEQ ID NO:4, the another polyunsaturated fatty acid produced according to the method recited above in connection with SEQ ID NO:4 and the final polyunsaturated fatty acid produced according to the 5 method described above in connection with SEQ ID NO:4.

Furthermore, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient the nutritional composition recited 10 with respect to SEQ ID NO:4 in an amount sufficient to effect the treatment or prevention.

The present invention also includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:5 (Figure 54). 15 Thus, the sequence may be that represented by SEQ ID NO:5. The sequence may encode a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate. It may also be derived from a mammal such as, for example, a mouse. The present invention also includes a purified protein encoded 20 by the nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of the protein.

Additionally, the invention also includes a method of producing an elongase enzyme, as described above, in which the nucleotide sequence isolated comprises either SEQ ID NO:5 or SEQ 25 ID NO:6. The host cell utilized may be as described above.

The present invention also encompasses a vector comprising:  
a) a nucleotide sequence comprising SEQ ID NO:5 (Figure 54) (or a  
30 nucleotide sequence comprising SEQ ID NO:6 (Figure 58)) operably linked to b) a promoter, as well as a host cell comprising this

vector. Again, the host cell may be as described above for the related methods using the other nucleotide sequences of the present invention.

Additionally, the invention includes a plant cell, plant or 5 plant tissue comprising the vector comprising SEQ ID NO:5 or 6, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. When the nucleotide sequence of the vector comprises SEQ ID NO:5, the polyunsaturated fatty 10 acid is selected from the group consisting of AA, ADA, GLA and STA. The invention also includes one or more plant oils or acids expressed by the plant cell, plant or plant tissue.

The present invention also includes a transgenic plant comprising the vector described above, wherein expression of the 15 nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Additionally, the present invention encompasses a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase, operably linked to a promoter, 20 wherein the DNA sequence comprises SEQ ID NO:5 (Figure 54) (or SEQ ID NO:6 (Figure 58)). Also, the invention includes a fluid produced by this transgenic, non-human mammal, wherein the fluid comprises a detectable level of at least one elongase or products thereof.

25 The invention also includes a method for producing a polyunsaturated fatty acid, similar to the methods described above, except that the isolated nucleotide sequence comprises SEQ ID NO:5 (Figure 54). The substrate polyunsaturated fatty acid may be selected from the group consisting of GLA, STA, AA, 30 ADA and ALA, and the product polyunsaturated fatty acid may be

selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA, respectively. The method may further comprise the step of exposing the expressed elongase enzyme to at least one desaturase in order to convert the

5 product polyunsaturated fatty acid to another polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group consisting of DGLA, 20:4n-3, ADA and  $\omega$ 6-docosapentaenoic acid, the another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-

10 docosapentaenoic acid and docosahexaenoic acid respectively, and the at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to production of  $\omega$ 6-docosapentaenoic acid, and  $\Delta$ 19-desaturase with respect to production of docosahexaenoic acid. The method may

15 further comprises the step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty

20 acid. The final polyunsaturated fatty acid may selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

The present invention also includes a nutritional composition comprising at least one polyunsaturated fatty acid

25 selected from the group consisting of the product polyunsaturated fatty acid produced according to the method above, the another polyunsaturated fatty acid is produced according to the method above, and the final polyunsaturated fatty acid produced according to the method above. The product

30 polyunsaturated fatty acid may be selected from the group

consisting of DGLA, 20:4n-3, ADA, and  $\omega$ 6-docosapentaenoic acid and STA. The another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid. The final polyunsaturated fatty acid 5 is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid. The nutritional composition may be selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

The present invention also includes a pharmaceutical 10 composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method above, the another polyunsaturated fatty acid produced according to the method above, and the final polyunsaturated fatty acid 15 produced according to the method above and 2) a pharmaceutically acceptable carrier.

Additionally, the present invention includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid 20 produced according to the method above, the another polyunsaturated fatty acid produced according to the method above and the final polyunsaturated fatty acid produced according to the method above. The product polyunsaturated fatty acid may be selected from the group consisting of DGLA, 25 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA. The another polyunsaturated fatty acid may be selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid. The final polyunsaturated fatty acid may 30 be selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

The invention includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the method above, the another polyunsaturated fatty acid produced 5 according to the method above and the final polyunsaturated fatty acid produced according to the method above.

Additionally, a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional 10 composition above in an amount sufficient to effect the prevention or treatment.

The present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:6 (Figure 58). 15 The isolated nucleotide sequence may comprise SEQ ID NO:6. The invention also includes a purified protein encoded by the nucleotide sequences.

Additionally, the present invention relates to an isolated nucleotide sequence corresponding to or complementary to at 20 least about 35% of the nucleotide sequence shown in SEQ ID NO:7 (Figure 72). This isolated sequence may be represented by SEQ ID NO:7. The sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid or a monounsaturated fatty acid as a substrate. In particular, the sequence may be 25 derived from a fungus of the genus Thraustochytrium and may specifically be isolated from Thraustochytrium aureum.

The present invention also includes a purified protein encoded by the above nucleotide sequence as well as a purified polypeptide which elongates polyunsaturated fatty acids or 30 monounsaturated fatty acids and has at least about 50% amino

acid similarity to the amino acid sequence of the purified protein encoded by the above nucleotide sequence.

Additionally, the present invention encompasses a method of producing an elongase enzyme comprising the steps of: a)

- 5 isolating the nucleotide sequence represented by SEQ ID NO:7 (Figure 72); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the elongase enzyme.

10 The host cell may be a eukaryotic cell or a prokaryotic cell.

The prokaryotic cell may be, for example an E. coli cell, a cyanobacterial cell, or a B. subtilis cell. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell. The fungal cell may be, for example,

15 Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. or Pichia spp. In particular, the fungal cell may be a yeast cell such as Saccharomyces spp., in particular, Saccharomyces cerevisiae,

20 Candida spp., Hansenula spp. or Pichia spp..

The invention also includes a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:7 (Figure 72) operably linked to b) a promoter, as well as a host cell comprising this vector. The host may be a prokaryotic cell or a

25 eukaryotic cell. Suitable examples of prokaryotic cells include E. coli, Cyanobacteria, and B. subtilis cells. Suitable examples of eukaryotic cells include a mammalian cell, an insect cell, a plant cell and a fungal cell. The fungal cell may be, for example, Saccharomyces spp., Candida spp., Lipomyces spp., Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and

Pichia spp. In particular, the fungal cell may be, for example, a yeast cell such as, for example, Saccharomyces spp., in particular, Saccharomyces cerevisiae, Candida spp., Hansenula spp. and Pichia spp.

5 The present invention includes a plant cell, plant or plant tissue comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of at least one fatty acid selected from the group consisting of a monounsaturated fatty acid and a polyunsaturated fatty acid by  
10 the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, dihomoo- $\gamma$ -linolenic acid (DGLA), 20:4n-3, and adrenic acid (ADA). The invention also includes one or more plant oils or fatty acids expressed by the plant cell, plant or plant tissue. Additionally, the present  
15 invention encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Furthermore, the present invention includes a transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:7 (Figure 72). The present invention also includes a fluid (e.g., milk) produced by the transgenic, non-human wherein the fluid comprises a detectable level of at least one elongase or products thereof such as, for example, DGLA,  $\omega$ 6-docosapentaenoic acid, ADA and/or 20:4n-3 (see Figure 1).

Additionally, the present invention includes a method for producing a polyunsaturated fatty acid comprising the steps of:  
30 a) isolating said nucleotide sequence represented by SEQ ID NO:7

(Figure 72); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of elongase enzyme encoded by the isolated nucleotide sequence; and d)

5 exposing the expressed elongase enzyme to a "substrate" polyunsaturated fatty acid in order to convert the substrate to a "product" polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be selected from the group consisting of, for example,  $\gamma$ -linolenic acid (GLA), stearidonic

10 acid (STA) and arachidonic acid (AA), and the product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA, respectively. The method may further comprise the step of exposing the product polyunsaturated fatty acid to at least one

15 desaturase in order to convert the product polyunsaturated fatty acid to "another" polyunsaturated fatty acid. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be selected from the group

20 consisting of, for example, AA, eicosapentaenoic acid (EPA),  $\omega$ 6-docosapentaenoic acid, respectively, and the at least one desaturase is  $\Delta$ 5-desaturase, with respect to production of AA or EPA, and  $\Delta$ 4-desaturase, with respect to production of  $\omega$ 6-docosapentaenoic acid. The method may further comprise the

25 step of exposing the another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert the another polyunsaturated fatty acid to a "final" polyunsaturated fatty acid. The final polyunsaturated fatty

acid may be, for example, docosahexaenoic acid (DHA), AA,  $\omega$ 6-docosapentaenoic acid, or  $\omega$ 3-docosapentaenoic acid.

Also, the present invention includes a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be selected from the group consisting of, for example, DGLA, 20:4n-3 and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid. The nutritional composition may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or an animal and may be administered enterally or parenterally. The nutritional composition may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, triglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, protein hydrolysates, sunflower oil, safflower oil, corn oil, and flax oil. It may also comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

Additionally, the present invention encompasses a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method and 2) a pharmaceutically acceptable carrier. The composition may be administered to a human or an animal. It may also further comprise at least one element selected from the group consisting of a vitamin, a mineral, a salt, a carbohydrate, an amino acid, a free fatty acid, a preservative, an excipient, an anti-histamine, a growth factor, an antibiotic, a diluent, a phospholipid, an antioxidant, and a phenolic compound. It may be administered enterally, parenterally, topically, rectally, intramuscularly, subcutaneously, intradermally, or by any other appropriate means.

The present invention also includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-described method, and the final polyunsaturated fatty acid produced according to the above-described method. The product polyunsaturated fatty acid may be, for example, DGLA, 20:4n-3, and ADA. The another polyunsaturated fatty acid may be, for example, AA, EPA, or  $\omega$ 6-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, DHA, adrenic acid,  $\omega$ 6-docosapentaenoic acid or  $\omega$ 3-docosapentaenoic acid.

Moreover, the present invention also includes a cosmetic

comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the above-described method, the another polyunsaturated fatty acid produced according to the above-  
5 described method, and the final polyunsaturated fatty acid produced according to the above-described method.

Additionally, the present invention includes a method of preventing or treating a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising  
10 administering to the patient the above nutritional composition in an amount sufficient to effect prevention or treatment.

All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

15

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents various fatty acid biosynthesis pathways. The role of the elongase enzyme (elo) should be noted.

Figure 2 represents the percent similarity and percent  
20 identity between the amino acid sequences of jojoba KCS and ELO2.

Figure 3 represents the S. cerevisiae ELO2 sequence homologous to the jojoba KCS sequence (primer sequence underlined) of Figure 2.

25 Figure 4A shows the physical map of pRAE-2 containing the MAELO cDNA. Figure 4B represents the physical map of the constitutive expression vector, pRAE-5, used for elongase enzyme production in yeast.

Figure 5 represents a comparison of the nucleotide  
30 sequences of clones pRAE-5 and pRAE-6.

Figure 6 illustrates the complete nucleotide sequence of Mortierella alpina elongase (MAELO).

Figure 7 represents the amino acid sequence of the Mortierella alpina elongase translated from MAELO (see Figure 5 6).

Figure 8 represents an amino acid sequence alignment among 3 elongases: S. cerevisiae ELO2 (GNS1), S. cerevisiae ELO3 (SUR4) and the translated MAELO sequence as shown in Figure 7.

Figure 9 represents a comparison between the nucleotide 10 sequence MAELO and the nucleotide sequence of ELO2 from S. cerevisiae.

Figures 10A and 10B represents the PUFA elongase activity of MAELO expressed in baker's yeast.

Figure 11 illustrates the PUFA elongase activity of MAELO 15 when co-expressed with the Δ5-desaturase cDNA from M. alpina to produce AA.

Figure 12 compares the PUFA elongase activity of MAELO to the overexpression of ELO2 from S. cerevisiae in baker's yeast.

Figures 13, 14 and 15 represent three separate comparisons 20 of amino acid sequences derived from C. elegans nucleotide sequences in the GenEMBL database with the translated MAELO.

Figure 16 shows the comparison between amino acid translations of two different mammalian sequences in the GenEMBL database and the translated MAELO.

25 Figure 17 shows the comparison of a translated DNA sequence (see published PCT application WO 88/07577) with the amino acid sequence derived from MAELO, which was detected during a database search.

Figure 18 shows the complete nucleotide sequence of the Δ5- 30 desaturase from M. alpina.

Figure 19 represents the initial GC-FAME analysis of MAD708 pool. The detection of a DGLA (C20:3n-6) peak should be noted.

Figure 20 represents the PUFA elongase activity of the five MAD708 clones in yeast with GLA as substrate. All clones have  
5 apparent elongase activity.

Figure 21 represents the DNA sequencing analysis of plasmid pRPB2. The analysis reveals an open reading frame of 957 bp in length.

Figure 22 shows the complete nucleotide sequence of the M.  
10 alpina cDNA, contained in the plasmid pRPB2, which is designated GLELO for its GLA elongase activity.

Figure 23 represents the amino acid sequence of the M.  
alpina elongase translated from GLELO (see Figure 22).

Figure 24 illustrates the n-6 PUFA elongase activity in an  
15 induced culture of 334(pRPB2) when supplemented with GLA.

Figure 25 represents the n-3 and n-6 PUFA elongase activity in an induced culture of 334(pRPB2) when supplemented with 25 µM of other fatty acid substrates.

Figure 26A illustrates the elongase activity of GLELO with  
20 GLA as a substrate when co-expressed with the M. alpina Δ5-desaturase cDNA to produce AA. Figure 26B illustrates the elongase activity of GLELO with STA as a substrate when co-expressed with the M. alpina Δ5-desaturase cDNA to produce EPA.

Figure 27 illustrates the comparison between the translated  
25 GLELO sequence (see Figure 23) and the translated MAELO sequence (see Figure 7).

Figure 28 represents a comparison of the amino acid sequence of 4 elongases: the translated amino acid sequence of GLELO (see Figure 23), MAELO (see Figure 7), S. cerevisiae ELO2

(GNS1), and S. cerevisiae ELO3 (SUR4). The histidine box is underlined.

Figure 29 represents an alignment between translated MAELO sequence and translated putative human homologue HS1 sequence.

5 Figure 30 represents an alignment between the translated MAELO sequence and the translated putative human homologue HS2 sequence.

10 Figure 31 shows an alignment between the translated MAELO sequence and the translated putative mouse homologue MM2 sequence.

Figure 32 represents an alignment between the translated MAELO and the translated putative mouse homologue AI225632 sequence.

15 Figure 33 illustrates an alignment between the translated GLELO sequence and the translated human homologue AI815960 sequence.

Figure 34 shows an alignment between the translated GLELO sequence and the translated putative human homologue HS1 sequence.

20 Figure 35 represents an alignment between the translated GLELO sequence and the translated putative human homologue sequence from AC004050.

25 Figure 36 illustrates an alignment between the translated GLELO sequence and the translated putative mouse homologue MM2 sequence.

Figure 37 represents an alignment of the translated GLELO sequence and a translated putative mouse homologue AI225632 sequence.

30 Figure 38 illustrates an alignment of the translated GLELO sequence and a translated putative mouse homologue U97107.

Figure 39 represents an alignment of the translated GLELO sequence and a translated putative C. elegans U68749 (F56H11.4) homologue sequence.

5 Figure 40 shows an alignment between the translated MAELO sequence and a translated putative C. elegans U68749 (F56H11.4) homologue sequence.

Figure 41 represents an alignment between the translated GLELO sequence and a translated putative Drosophila melanogaster homologue sequence, DM1.

10 Figure 42 illustrates an alignment between the translated MAELO sequence and a translated putative Drosophila melanogaster homologue sequence, DM1.

Figure 43 illustrates the complete nucleotide sequence of a human elongase HSELO1.

15 Figure 44 represents the deduced amino acid sequence of the human elongase HSELO1.

Figure 45 illustrates the elongase activity (PUFA and others) of an induced culture of 334(pRAE-58-A1) when supplemented with GLA or AA.

20 Figure 46 shows the complete nucleotide sequence of the C. elegans elongase CEELO.

Figure 47 represents the deduced amino acid of C. elegans elongase CEELO.

25 Figure 48 illustrates the PUFA elongase activity of an induced culture of 334(pRET-21) and 334(pRET-22) when supplemented with GLA and AA.

Figure 49 represents the complete nucleotide sequence of the putative human elongase gene HS3.

30 Figure 50 illustrates the deduced amino acid sequence of the putative human elongase enzyme HS3.

Figure 51 represents the elongase activity (PUFA and others) of HSEL01 expressed in baker's yeast when supplemented with GLA, AA, STA, EPA, OA, or ALA.

5 Figure 52 represents the elongase activity (PUFA and others) of HSEL01 expressed in baker's yeast when supplemented with 25 mM or 100 mM of GLA, AA, or EPA.

Figures 53A, 53B, and 53C represent the elongase activity (PUFA and others) of HSEL01 expressed in baker's yeast when supplemented with PA, SA, ARA, BA, PTA, OA, EA, LA, GLA, DGLA, 10 AA, ADA, ALA, STA, EPA, or DPA, or when no substrate is present.

Figure 54 represents the complete nucleotide sequence of mouse elongase MELO4.

Figure 55 represents the deduced amino acid sequence of the mouse elongase MELO4.

15 Figure 56 represents the PUFA elongase activity of MELO4 expressed in baker's yeast when supplemented with GLA, AA, ADA, STA, EPA, or DPA.

Figures 57A, 57B, and 57C represent the PUFA elongase activity of MELO4 expressed in baker's yeast when supplemented 20 with PA, SA, ARA, BA, PTA, OA, EA, LA, GLA, DGLA, AA, ADA, ALA, STA, EPA, or DPA, or when no substrate is present.

Figure 58 represents the complete nucleotide sequence of mouse elongase MELO7.

Figure 59 represents the deduced amino acid sequence of the 25 mouse elongase MELO7.

Figure 60 represents the elongase activity (PUFA and others) of MELO7 expressed in baker's yeast when supplemented with GLA, AA, ADA, STA, EPA, or DPA.

Figure 61 shows the activity of the C. elegans elongase 30 when expressed in yeast when no substrate is present and with addition of AA or GLA.

Figure 62 illustrates the PUFA elongase activity of an induced culture of 334(pRET22) when supplemented with 50 µM of various substrates.

Figure 63 represents the PUFA elongase activity with GLA  
5 (Figure 63A) or STA (Figure 63B) as a substrate when co-expressed with the M. alpina Δ5-desaturase cDNA to produce AA or EPA, respectively.

Figure 64 represents an amino acid sequence alignment among 4 elongases: HSELO1 (Figure 44), MELO4 (Figure 55), GLELO  
10 (Figure 23), and CEELO (Figure 47).

Figure 65 represents the consensus nucleotide sequence of the partial Thraustochytrium aureum (T. aureum) 7091 elongase gene.

Figure 66 represents the deduced amino acid sequence of the  
15 T. aureum 7091 elongase gene in Figure 65.

Figure 67 represents the complete nucleotide sequence of the T. aureum 7091 elongase TEL01, from plasmid pRAT-4-A1.

Figure 68 represents the complete nucleotide sequence of the T. aureum 7091 elongase TEL01, from plasmid pRAT-4-A2.

20 Figure 69 represents the complete nucleotide sequence of the T. aureum 7091 elongase TEL01, from plasmid pRAT-4-A3.

Figure 70 represents the complete nucleotide sequence of the T. aureum 7091 elongase TEL01, from plasmid pRAT-4-A4.

Figure 71 represents the complete nucleotide sequence of  
25 the T. aureum 7091 elongase TEL01, from plasmid pRAT-4-A6.

Figure 72 represents the complete nucleotide sequence of the T. aureum 7091 elongase TEL01, from plasmid pRAT-4-A7.

Figure 73 represents the complete nucleotide sequence of the T. aureum 7091 elongase TEL01, from plasmid pRAT-4-D1.

Figure 74 represents the deduced amino acid sequence of the T. aureum 7091 elongase TELO1, from plasmid pRAT-4-A1.

Figure 75 represents the deduced amino acid sequence of the T. aureum 7091 elongase TELO1, from plasmid pRAT-4-A2.

5 Figure 76 represents the deduced amino acid sequence of the T. aureum 7091 elongase TELO1, from plasmid pRAT-4-A3.

Figure 77 represents the deduced amino acid sequence of the T. aureum 7091 elongase TELO1, from plasmid pRAT-4-A4.

10 Figure 78 represents the deduced amino acid sequence of the T. aureum 7091 elongase TELO1, from plasmid pRAT-4-A6.

Figure 79 represents the deduced amino acid sequence of the T. aureum 7091 elongase TELO1, from plasmid pRAT-4-A7.

Figure 80 represents the deduced amino acid sequence of the T. aureum 7091 elongase TELO1, from plasmid pRAT-4-D1.

15 Figure 81 represents the elongase activity of TELO1 expressed in baker's yeast when supplemented with GLA or EPA.

Figure 82 represents an amino acid sequence alignment among the TELO1 elongases from the 7 plasmids: pRAT-4-A1 (Figure 74), pRAT-4-A2 (Figure 75), pRAT-4-A3 (Figure 76), pRAT-4-A4 (Figure 20 77), pRAT-4-A6 (Figure 78), pRAT-4-A7 (Figure 79), and pRAT-4-D1 (Figure 80).

Figure 83 represents an alignment between the translated TELO1 sequence and the translated HSELO1 sequence.

25 Figure 84 represents an alignment between the translated TELO1 sequence and the translated MELO4 sequence.

Figure 85 represents an alignment between the translated TELO1 sequence and the translated GLELO sequence.

Figure 86 represents an alignment between the translated TELO1 sequence and the translated CEELO sequence.

Figure 87 represents the elongase activity of TELO1 expressed in baker's yeast when supplemented with GLA, AA, STA, EPA, or no substrate.

Figure 88 represents the elongase activity of TELO1  
5 expressed in baker's yeast when supplemented with LA, ALA, GLA,  
STA, DGLA, ETA, AA or EPA.

#### DETAILED DESCRIPTION OF THE INVENTION

10 The subject invention relates to nucleotide and corresponding amino acid sequences of two elongase cDNAs derived from Mortierella alpina, as well as to nucleotide and corresponding amino acid sequences of an elongase cDNA derived from a human, an elongase cDNA derived from C. elegans, two  
15 elongase cDNAs derived from a mouse, and an elongase cDNA derived from Thraustochytrium aureum. Furthermore, the subject invention also includes uses of the cDNAs and of the proteins encoded by the genes. For example, the genes and corresponding enzymes may be used in the production of polyunsaturated fatty  
20 acids and/or monounsaturated fatty acids such as, for example, DGLA, AA, ADA, EPA and/or DHA which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, cosmetics, and to other valuable products.

25 The Elongase Genes and Enzymes Encoded Thereby

As noted above, an elongase enzyme encoded by an elongase cDNA is essential in the production of various polyunsaturated fatty acids, in particular, 20-24 carbon PUFAs. With respect to the present invention, the nucleotide sequence of the isolated  
30 M. alpina elongase cDNA (MAELO) is shown in Figure 6, and the amino acid sequence of the corresponding purified protein or

enzyme encoded by this nucleotide sequence is shown in Figure 7. Additionally, the nucleotide sequence of the isolated GLA elongase cDNA (GLELO) is shown in Figure 22, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this nucleotide sequence is shown in Figure 23. The nucleotide sequence of the isolated human sequence 1 (HSEL01) elongase is shown in Figure 43, and the amino acid sequence of the corresponding purified protein or enzyme encoded by this sequence is shown in Figure 44. Furthermore, the nucleotide sequence of the isolated *C. elegans* elongase cDNA (CEEL01) is shown in Figure 46, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 47. Additionally, the nucleotide sequence of the isolated mouse PUFA elongation enzyme (MEL04) is shown in Figure 54, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 55. Moreover, the nucleotide sequence of the second isolated mouse PUFA elongation enzyme (MEL07) is shown in Figure 58, and the amino acid sequence of the corresponding purified protein or enzyme encoded thereby is shown in Figure 59. Also, the nucleotide sequence of the isolated *T. aureum* elongase cDNA (TELO1) is shown in Figure 72, and the amino acid sequence of the corresponding purified protein of enzyme encoded thereby is shown in Figure 79.

As an example, several of the isolated elongases encoded by the cDNAs of the present invention elongate GLA to DGLA or elongate STA to 20:4n-3 or elongate AA to ADA. The production of arachidonic acid from DGLA, or EPA from 20:4n-3, is then catalyzed by, for example, a Δ5-desaturase. Thus, neither AA (or EPA), nor DGLA (or 20:4n-3) nor ADA (or ω3-docosapentaenoic

acid), can be synthesized without at least one elongase cDNA and enzyme encoded thereby.

It should be noted that the present invention also encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 50%, preferably at least about 60%, and more preferably at least about 70% of the nucleotides in SEQ ID NO:1 (i.e., the nucleotide sequence of the MAELO cDNA described herein (see Figure 6)). Furthermore, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:2 (i.e., the nucleotide sequence of the GLELO cDNA described herein (see Figure 22)). Additionally, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:3 (i.e., the nucleotide sequence of the human sequence 1 (HSEL01) cDNA described herein (see Figure 43)). In addition, the present invention also includes nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:4 (i.e., the nucleotide sequence of the C. elegans cDNA, CEELO1, described herein (see Figure 46)). Further, the present invention also includes nucleotide sequences (and the corresponding encoded

proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:5 or SEQ ID NO:6 (i.e., the 5 nucleotide sequence of the mouse PUFA elongases MELO4 and MELO7, described herein (see Figures 54 and 58, respectively)). Also, the present invention encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to (i.e., having identity to) or complementary to at least about 10 35%, preferably at least about 45%, and more preferably at least about 55% of the nucleotides in SEQ ID NO:7 (i.e., the nucleotide sequence of the T. aureum elongase gene TELO1, described herein (see Figure 72)). It should be noted that the "most preferable" range, referred to in each instance, may be 15 increased by increments of ten percent. For example, if "at least 55%" is the most preferable range recited above, with respect to a particular sequence, such a range also naturally includes "at least 65% identity", "at least 75% identity", "at least 85% identity", and "at least 95% identity".  
20 The corresponding or complementary sequences may be derived from non-Mortierella sources or sources other than from which the isolated, original sequences were derived (e.g., a eukaryote (e.g., Thraustochytrium spp. (e.g., Thraustochytrium aureum and Thraustochytrium roseum), Schizochytrium spp. (e.g.,  
25 Schizochytrium aggregatum), Conidiobolus spp. (e.g., Conidiobolus nanodes), Entomophthora spp. (e.g., Entomophthora exitalis), Saprolegnia spp. (e.g., Saprolegnia parasitica and Saprolegnia diclina), Leptomyces spp. (e.g., Leptomyces lacteus), Entomophthora spp., Pythium spp., Porphyridium spp.  
30 (e.g., Porphyridium cruentum), Conidiobolus spp., Phytophthora spp., Penicillium spp., Coidosporium spp., Mucor spp. (e.g.,

Mucor circinelloides and Mucor javanicus), Fusarium spp.,  
Aspergillus spp., Rhodotorula spp., Amphidinium carteri,  
Chaetoceros calcitrans, Cricosphaera carterae, Cryptothecodinium  
cohnii, Cryptomonas ovata, Euglena gracilis, Gonyaulax polyedra,  
5 Gymnodinium spp. (e.g. Gymnodinium nelsoni), Gyrodinium cohnii,  
Isochrysis spp. (e.g. Isochrysis galbana), Microalgae MK8805,  
Nitzschia frustulum, Pavlova spp. (e.g. Pavlova lutheri),  
Phaeodactylum tricornutum, Prorocentrum cordatum, Rhodomonas  
lens, and Thalassiosira pseudonana), a Psychrophilic bacteria  
10 (e.g., Vibrio spp. (e.g., Vibrio marinus)), a yeast (e.g.,  
Dipodascopsis uninucleata), a non-mammalian organism such as a  
fly (e.g., Drosophila melanogaster) or Caenorhabditis spp.  
(e.g., Caenorhabditis elegans), or a mammal (e.g., a human or a  
mouse). Such sequences may also be derived from a species  
15 within the genus Mortierella, other than the species alpina, for  
example, Mortierella elongata, Mortierella exigua, Mortierella  
isabellina, Mortierella hygrophila, and Mortierella ramanniana,  
va. angulispora.

Furthermore, the present invention also encompasses  
20 fragments and derivatives of the nucleotide sequences of the  
present invention (i.e., SEQ ID NO:1 (MAELO), SEQ ID NO:2  
(GLELO), SEQ ID NO:3 (HSELO1), SEQ ID NO:4 (CEELO1)), SEQ ID  
NO:5 (MELO4), SEQ ID NO:6 (MEL07) and SEQ ID NO:7 (TEL01)) as  
well as of the corresponding sequences derived from non-  
25 Mortierella or non-mammalian sources, etc., as described above,  
and having the above-described complementarity or  
correspondence/identity to the 7 sequences. Functional  
equivalents of the above-sequences (i.e., sequences having  
elongase activity) are also encompassed by the present  
30 invention.

For purposes of the present invention, "complementarity" is defined as the degree of relatedness between two DNA segments. It is determined by measuring the ability of the sense strand of one DNA segment to hybridize with the antisense strand of the 5 other DNA segment, under appropriate conditions, to form a double helix. In the double helix, wherever adenine appears in one strand, thymine appears in the other strand. Similarly, wherever guanine is found in one strand, cytosine is found in the other. The greater the relatedness between the nucleotide 10 sequences of two DNA segments, the greater the ability to form hybrid duplexes between the strands of two DNA segments.

"Identity" between two nucleotide sequences is defined as the degree of sameness, correspondence or equivalence between the same strands (either sense or antisense) of two DNA 15 segments. The greater the percent identity, the higher the correspondence, sameness or equivalence between the strands.

"Similarity" between two amino acid sequences is defined as the presence of a series of identical as well as conserved amino acid residues in both sequences. The higher the degree of 20 similarity between two amino acid sequences, the higher the correspondence, sameness or equivalence of the two sequences. ("Identity" between two amino acid sequences is defined as the presence of a series of exactly alike or invariant amino acid residues in both sequences.)

25 The definitions of "complementarity", "identity", and "similarity" are well known to those of ordinary skill in the art.

The invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and 30 has at least about 50%, preferably at least about 70%, and more preferably at least about 90% amino acid similarity to the amino

acid sequences of the above-noted proteins (see, e.g., Figure 7 (MAEL0)) and which are, in turn, encoded by the above-described nucleotide sequences. Additionally, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30%, preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 23 (GLELO)) and which are, in turn, encoded by the above-described nucleotide sequences.

10 Furthermore, the invention also includes a purified polypeptide which elongates polyunsaturated and monounsaturated fatty acids and has at least about 30%, preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g.,

15 Figure 44 (HSELO1)) and which are, in turn, encoded by the above-described nucleotide sequences. Also, the present invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30%, preferably at least about 60%, and more preferably at least

20 about 90% amino acid similarity to the amino acid sequences of the above-noted proteins (see, e.g., Figure 47 (CEEL01)) and which are, in turn, encoded by the above-described nucleotide sequences. The present invention also includes a purified polypeptide which elongates polyunsaturated fatty acids and has

25 at least about 30%, preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above noted proteins (see, e.g., Figure 55 (MEL04) and Figure 58 (MEL07)) and which are, in turn, encoded by the above-described nucleotide sequences. Also, the present

30 invention includes a purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30%,

preferably at least about 60%, and more preferably at least about 90% amino acid similarity to the amino acid sequences of the above noted proteins (see, e.g., Figure 79 (TEL01)) and which are, in turn, encoded by the above-described nucleotide 5 sequences.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA elongase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence corresponding or 10 complementary to the nucleotide sequence represented by SEQ ID NO:1 shown in Figure 6 (MAELO) and/or SEQ ID NO:2 shown in Figure 22 (GLELO) and/or SEQ ID NO:3 (HSELO1) shown in Figure 43 and/or SEQ ID NO:4 (CEEL01) shown in Figure 46 and/or SEQ ID NO:5 (MELO4) shown in Figure 54 and/or SEQ ID NO:6 (MEL07) shown 15 in Figure 58 and/or SEQ ID NO:7 (TEL01) shown in Figure 72. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see 20 Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain 25 complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementarity. Such variables are well known in the art. 30 More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value

of T<sub>m</sub>, melting temperature, for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating T<sub>m</sub> have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

Production of the Elongase Enzyme

Once the gene encoding the elongase has been isolated, it may then be introduced into either a prokaryotic or eukaryotic host cell through the use of a vector, plasmid or construct.

The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the elongase as well as any promoter which is functional in the host cell and is able to elicit expression of the elongase encoded by the nucleotide sequence. The promoter is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects transcription or expression of the coding sequence.) Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucoisomerase, phosphoglycerate kinase, acid phosphatase, T7, TP1, lactase, metallothionein, cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T-

antigen, ovalalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired expression products as well as the nature of the host cell.

- As noted above, once the vector has been constructed, it
- 5 may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for example, transfection, transformation and electroporation (see Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)).
- 10 The host cell is then cultured under suitable conditions permitting expression of the PUFA which is then recovered and purified.

It should also be noted that one may design a unique triglyceride or oil if one utilizes one construct or vector

15 comprising the nucleotide sequences of two or more cDNAs (e.g., MAELO, GLELO, HSELO1, CEELO1 and/or TELO1). This vector may then be introduced into one host cell. Alternatively, each of the sequences may be introduced into a separate vector. These vectors may then be introduced into two host cells,

20 respectively, or into one host cell.

Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells include, for

25 example, mammalian cells, plant cells, yeast cells such as Saccharomyces spp., Lipomyces spp., Candida spp. such as Yarrowia (Candida) spp., Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such as filamentous fungal cells, for example, Aspergillus, Neurospora

30 and Penicillium. Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, the site of the construct's integration can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

A transgenic mammal may also be used in order to express the enzyme of interest (i.e., the elongase) encoded by one or both of the above-described nucleotide sequences. More specifically, once the above-described construct is created, it may be inserted into the pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al., Science 278:2130-2133 (1997)). Gestation and birth are then

permitted to occur (see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFAs, as compared to the levels normally found in the non-transgenic animal. Subsequent generations may be monitored for production of the altered or enhanced levels of PUFAs and thus incorporation of the gene or genes encoding the elongase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse, a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the ability to incorporate DNA encoding the enzyme of interest into its genome.

For expression of an elongase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the elongase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific regulatory sequence such as those of U.S. Patent Nos. 5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of an elongase gene or genes, or antisense

elongase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The elongase polypeptide coding region may be expressed either by itself or with other genes, in order to produce tissues  
5 and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' region of the gene from which the initiation region was obtained  
10 or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected as a matter of convenience rather than because of any particular property.

15 As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)), plant cell, plant tissue, corn, potatoe, sunflower, safflower or flax may also be utilized as a host or host cell, respectively, for expression of the elongase enzyme(s) which may, in turn, be utilized in the production of  
20 polyunsaturated fatty acids. More specifically, desired PUFAs can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing a source for PUFAs, seed oil components may be manipulated through the expression of the elongase genes, as well as perhaps desaturase  
25 genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA sequence encoding the elongase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and  
30 under conditions sufficient for expression of the elongase gene. The vector may also comprise one or more genes which encode

other enzymes, for example,  $\Delta$ 4-desaturase,  $\Delta$ 5-desaturase,  $\Delta$ 6-desaturase,  $\Delta$ 8-desaturase,  $\Delta$ 9-desaturase,  $\Delta$ 10-desaturase,  $\Delta$ 12-desaturase,  $\Delta$ 13-desaturase,  $\Delta$ 15-desaturase,  $\Delta$ 17-desaturase and/or  $\Delta$ 19-desaturase. The plant tissue or plant may produce 5 the relevant substrate (e.g., DGLA, GLA, STA, AA, ADA, EPA, 20:4n-3, etc.) upon which the enzymes act or a vector encoding enzymes which produce such substrates may be introduced into the plant tissue, plant cell, plant, or host cell of interest. In addition, substrate may be sprayed on plant tissues expressing 10 the appropriate enzymes. Using these various techniques, one may produce PUFAs (e.g., n-6 unsaturated fatty acids such as DGLA, AA or ADA, or n-3 fatty acids such as EPA or DHA) by use of a plant cell, plant tissue, plant, or host cell of interest. It should also be noted that the invention also encompasses a 15 transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

The substrates which may be produced by the host cell 20 either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector, which is subsequently introduced into the host cell, are shown in Figure 1.

In view of the above, the present invention also 25 encompasses a method of producing one of the elongase enzymes described above comprising the steps of: 1) isolating the desired nucleotide sequence of the elongase cDNA; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said vector into a host cell under time and 30 conditions sufficient for the production of the elongase enzyme.

The present invention also encompasses a method of producing polyunsaturated fatty acids comprising exposing an acid to the elongase(s) produced as above such that the elongase converts the acid to a polyunsaturated fatty acid. For example,

5 when GLA is exposed to elongase, it is converted to DGLA. DGLA may then be exposed to  $\Delta 5$ -desaturase which converts the DGLA to AA. The AA may then be converted to EPA by use of  $\Delta 17$ -desaturase which may be, in turn, converted to DHA by use of elongase and a  $\Delta 4$ -desaturase. Alternatively, elongase may be

10 utilized to convert 18:4n-3 to 20:4n-3 which may be exposed to  $\Delta 5$ -desaturase and converted to EPA. Elongase may also be used to convert 18:3n-3 to 20:3n-3, which may be, in turn, converted to 20:4n-3 by a  $\Delta 8$ -desaturase. Thus, elongase may be used in the production of polyunsaturated fatty acids which may be used,

15 in turn, for particular beneficial purposes. (See Figure 1 for an illustration of the many critical roles the elongase enzyme plays in several biosynthetic pathways.)

#### Uses of the Elongase Gene and Enzyme Encoded Thereby

20 As noted above, the isolated elongase cDNAs and the corresponding elongase enzymes (or purified polypeptides) encoded thereby have many uses. For example, each cDNA and corresponding enzyme may be used indirectly or directly in the production of polyunsaturated fatty acids, for example, DGLA, AA, ADA, 20:4n-3 or EPA. ("Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter of which is utilized in a composition (e.g., the conversion of GLA to DGLA)). "Indirectly" is meant to encompass the situation where a fatty acid is converted to

25 another fatty acid (i.e., a pathway intermediate) by elongase

30

(e.g., GLA to DGLA) and then the latter fatty acid is converted to another fatty acid by use of a non-elongase enzyme (e.g., DGLA to AA by  $\Delta 5$ -desaturase)). These polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the elongase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses are described, in detail, below.

10 Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including 15 for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention 20 comprises at least one oil or acid produced by use of at least one elongase enzyme, produced using the respective elongase gene, and may either be in a solid or liquid form.

Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use.

25 The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

30 Examples of macronutrients which may be added to the composition include but are not limited to edible fats,

- carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.
- With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulae, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

The nutritional composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food

of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or fatty acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form,

the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and, more preferably, less than 660 mOsm.

5       The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the PUFAs produced in accordance with the present invention. The presence of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to  
10      the provision of PUFAs, it may also be desirable to add zinc, copper, folic acid and antioxidants to the composition. It is believed that these substance boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may  
15      also be supplemented with these elements.

In a more preferred embodiment, the nutritional composition comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight % of the carbohydrate is indigestible oligosaccharide. In a more  
20      preferred embodiment, the nutritional composition additionally comprises protein, taurine, and carnitine.

As noted above, the PUFAs produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant  
25      formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or disease states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% as EPA,  
30      from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus,

fatty acids such as DGLA, AA, EPA and/or docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of AA, DGLA and GLA. More preferably, the oil blend will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10%

conversion rate of DGLA to AA can be used to produce an AA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% to 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal,

5 regulating the timing, extent and specificity of elongase expression, as well as the expression of other desaturases, can be used to modulate PUFA levels and ratios. The PUFAs/acids produced in accordance with the present invention (e.g., AA and DGLA) may then be combined with other PUFAs/acids (e.g., GLA) in

10 the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal

15 consumption.

#### Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the fatty acids and/or resulting oils produced using at least one of the elongase cDNAs (i.e., MAELO, GLELO, HSELO1, CEELO, MELO4 and MELO7), in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or

powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include

5 isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

10 Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these  
15 substances.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose,  
20 sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with  
25 antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as  
30 Intralipids™. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA,

and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the 5 formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical 10 compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with 15 physiologically acceptable diluents, preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or 20 burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, 25 age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

The present invention also includes the treatment of 30 various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the

compositions of the present invention may be used to treat restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may also be treated with the compositions of the invention.

- 5 Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

10 Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions. Addition of fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may 15 also be useful for treating cachexia associated with cancer.

The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have been demonstrated in 20 diabetic animals.

Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the elongase enzyme(s), may also be used in the treatment of eczema, in the reduction of blood pressure, and in 25 the improvement of mathematics examination scores.

Additionally, the compositions of the present invention may be used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue 30 (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976), reduction or prevention of gastrointestinal bleeding and other

side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after 5 viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

10 Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

15 Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as well as humans, as 20 animals experience many of the same needs and conditions as humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in animal topical ointments.

The present invention may be illustrated by the use of the 25 following non-limiting examples:

Example I

Determination of Codon Usage in *Mortierella alpina*

The 5' end of 1000 random cDNA clones were sequenced from Mortierella alpina cDNA library. The sequences were translated in six reading frames using GCG (Genetics Computer Group (Madison, Wisconsin)) with the FastA algorithm (Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444-2448 (1988)) to search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein), specifically with the Swissprot database (GeneBio, Geneva, Switzerland). Many of the clones were identified as a putative housekeeping gene based on protein sequence homology to known genes. Twenty-one M. alpina cDNA sequences which matched with known, housekeeping genes in the database were selected (see Table 1 below). M. alpina codon bias table (see Table 2) was generated based on these 21 sequences as well as the full length M. alpina Δ5-, (see Figure 18), Δ6-, and Δ12-desaturase sequences. Since the FastA alignment between the putative protein coded by the M. alpina cDNA sequence and the known protein sequence was weak in some areas, only the codons from areas of strong homology were used.

Table 1

Clone #	Match	# of bp	# of
193	Elongation factor 1-alpha	426	14
143	60S ribosomal protein L17	417	13
235	Actin I	360	12
299	40S ribosomal protein YS11	387	12
390	Ras-related protein rab-1a	342	11
65	40S ribosomal protein RP10	366	12
289	Ubiquitin-conjugating enzyme E2-16 KD	294	9
151	Ubiquinol-cytochrome C reductase	375	12
80	Initiation factor 5A-2	183	6
33	60S ribosomal protein L15	252	8

132	60S ribosomal protein L3-2	300	10
198	Histone H3	285	9
286	6-phosphogluconate dehydrogenase, decarboxylating	363	12
283	40S ribosomal protein S22	261	8
127	Elongation factor 2	231	7
197	Actin, gamma	252	8
496	40S ribosomal protein S16	270	9
336	Histone H4	219	7
262	Ubiquitin	228	7
188	Guanine nucleotide-binding protein beta subunit-like protein	213	7
81	Ubiquitin	228	7
21	TOTAL	6252	20

Table 2

5

	Amino acid	Codon Bias	% used	Amino acid	Codon Bias	% used
	Ala	GCC	63%	Lys	AAG	96%
	Arg	CGC	50%	Met	ATG	100%
	Asn	AAC	97%	Phe	TTC	78%
10	Asp	GAC	65%	Pro	CCC	68%
	Cys	TGC	87%	Ser	TCC	46%
	Gln	CAG	78%	Thr	ACC	78%
	Glu	GAG	85%	Trp	TGG	100%
	Gly	GGT	47%	Tyr	TAC	95%
15	His	CAC	91%	Val	GTC	72%
	Ile	ATC	72%	Stop	TAA	50%
	Leu	CTC	49%			

20

Example IICloning of a Full-length Elongase-like cDNA from *M. alpina*

The  $\beta$ -ketoacyl-coenzyme A synthase (KCS) from jojoba  
25 and the *Saccharomyces cerevisiae* elongase (ELO2) were  
aligned to determine an area of amino acid homology (see  
Figure 2). The codon bias was applied to the area of

sequence corresponding to the homologous amino acids between the two elongases, and primers were designed based on this biased sequence (see Figure 3). The cDNA was excised from the M11 M. alpina cDNA library (Knutzon et al., J. Biol. Chem. 273:29360-29366 (1998)), which contains approximately  $6 \times 10^5$  clones with an average insert size of 1.1 Kb. The excised cDNA was amplified with internal primer RO339 (5' -TTG GAG AGG AGG AAG CGA CCA CCG AAG ATG ATG- 3') and a vector forward primer RO317 (5' - CAC ACA GGA AAC AGC TAT GAC CAT GAT TAC G - 3'). Polymerase Chain Reaction (PCR) was carried out in a 100  $\mu$ l volume containing: 300 ng of excised M. alpina cDNA library, 50 pmole each primer, 10  $\mu$ l of 10X buffer, 1  $\mu$ l 10 mM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 1.0 U of Taq Polymerase. Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94°C for 2 mins., then 30 cycles of 94°C for 1 min., 58°C for 2 mins., and 72°C for 3 mins. PCR was followed by an additional extension at 72°C for 7 minutes.

The PCR amplified product was run on a gel, an amplified fragment of approximately 360 bp was gel purified, and the isolated fragment was directly sequenced using ABI 373A DNA Sequencer (Perkin Elmer, Foster City, CA). The sequence analysis package of GCG was used to compare the obtained sequence with known sequences. The sequence was translated in all six reading frames in the GCG Analysis Program using the FastA algorithm (Pearson and Lipman, supra). The Swissprot database (GeneBio, Geneva, Switzerland) of proteins was

searched. This translated cDNA fragment was identified as a part of a putative elongase based on the homology of the putative protein sequence to the S. cerevisiae ELO2 (GNS1), having 41.3% identity in 63 amino acids.

5 New primers were designed based on the putative elongase sequence and the vector, pZL1 (Life Technologies, Inc., Gaithersburg, MD) sequence used to construct M. alpina cDNA library. The M. alpina excised cDNA library was PCR amplified again using primers RO350  
10 (5' -CAT CTC ATG T CCG CCA TGG CCG CCG CAA TCT TG- 3'), which has an added *BamHI* restriction site (underlined), and the vector reverse primer RO352 (5' -ACG CGT ACG TAA AGC TTG- 3') to isolate the full length M. alpina elongase cDNA, using previously described conditions.  
15 The termini of the approximately 1.5 Kb PCR amplified fragment was filled-in with T4 DNA polymerase (Boehringer Mannheim Corp., Indianapolis, IN) to create blunt ends and cloned into the pCR-blunt vector (Invitrogen Corp., Carlsbad, CA). This resulted in two clones, pRAE-1 and  
20 pRAE-2 (see Figure 4A). (Plasmid DNA pRAE-2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, on August 28, 1998, under the terms of the Budapest Treaty, and was accorded deposit number ATCC 203166.) The  
25 elongase cDNAs from these vectors were cut out as an *EcoRI* fragment and cloned into the *EcoRI* digested pYX242 (Novagen, Madison, WI) vector. The clones pRAE-5 and pRAE-6 (see Figure 4B) have the elongase cDNAs from pRAE-1 and pRAE-2, respectively. (Plasmid DNA pRAE-5 was  
30 deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-

2209, on August 28, 1998, under the terms of the Budapest  
Treaty, and was accorded deposit number ATCC 203167.)  
The sequencing of pRAE-5 and pRAE-6 revealed that 5'  
untranslated region of the elongase gene in pRAE-5 is 16  
5 bp shorter than that in pRAE-6 (see Figure 5). The  
complete *M. alpina* elongase cDNA sequence, designated  
MAELO was obtained from pRAE-2 (see Figure 6). Figure 7  
is the amino acid sequence obtained from the translation  
of MAELO. The Swissprot database (GeneBio, Geneva,  
10 Switzerland) was searched again, as previously described,  
with the translated MAELO: MAELO has 44.3% identity in  
317 amino acids with *S. cerevisiae* GNS1(ELO2) and 44.7%  
identity in 318 amino acids with *S. cerevisiae*  
SUR4(ELO3). The FastA alignment among the three  
15 elongases is shown in Figure 8. At the nucleotide level  
(see Figure 9), MAELO has 57.4% identity in 549 bp  
overlap with *S. cerevisiae* GNS1(ELO2) (GenBank Accession  
# S78624). However, the identity between the complete  
MAELO gene of 954 bp and *S. cerevisiae* GNS1(ELO2) is  
20 33.0%.

### Example III

#### Expression of *M. alpina* Elongase cDNA in Baker's Yeast

25 The constructs pRAE-5, and pRAE-6 were transformed  
into *S. cerevisiae* 334 (Hoveland et al., *Gene* 83:57-64  
(1989)) and screened for elongase activity. The plasmid  
pCGN7875 (Calgene LLC, Davis, CA) containing jojoba KCS  
gene in pYES2 vector (Invitrogen Corp., Carlsbad, CA) was  
30 used as a positive control. The substrate used to detect  
elongase activity in *M. alpina* elongase (MAELO) was GLA

and that in jojoba KCS was oleic acid (OA). The negative control strain was S. cerevisiae 334 containing pYX242 vector. The cultures were grown for 40-48 hours at 25°C, in selective media (Ausubel et al., Short Protocols in Molecular Biology, Ch. 13, p. 3-5 (1992)), in the presence of a particular substrate. The expression of the jojoba KCS gene cloned in pYES2 was under the control of GAL1 promoter, while the promoter in pYX242 is TP1, which is constitutive. Hence, the 334(pCGN7875) and 334(pYES2) cultures were induced with galactose. The GC-FAME analysis of the lipid fraction of each cell pellet was performed as previously described (Knutzon et al., supra).

The elongase activity results from different experiments are provided in Figure 10A and 10B. The jojoba KCS elongates long chain monounsaturated fatty acids 18:1n-9 to 20:1n-9. The amino acid homology between the M. alpina elongase (MAELO) and the S. cerevisiae ELO2 and ELO3 suggested that the proteins encoded by these genes may have similar substrate specificity. The activity of the M. alpina elongase, elongation (MAELO) of long chain monounsaturated and saturated fatty acids, is seen in the conversion of 18:1n-9 to 20:1n-9 and also in the synthesis of 24:0. The control strain, 334(pYX242) has very little or no detectable amount of 20:1 and 24:0 (see Figure 10A). M. alpina elongase (MAELO) also acts on at least one PUFA, converting 18:3n-6(GLA) to 20:3n-6(DGLA). The percentage of the 20:3n-6 in total lipid is higher in the strain 334(pRAE-5) and 334(pRAE-6) with the M. alpina elongase (MAELO) cDNA when compared to that in the control

334(pYX242). The percentages of 20:3n-6 produced were 0.092% for 334(pYX242) vs. 0.324% for 334(pRAE-5) and 0.269% for 334(pRAE-6) (shown in parenthesis in Figures 10A and 10B). This difference in the fatty acid profile  
5 is also seen in the total amount of 20:3n-6 produced. Only 0.226  $\mu$ g of 20:3n-6 was produced by 334(pYX242) while 334(pRAE-5) and 334(pRAE-6) produced 2.504  $\mu$ g of 20:3n-6 and 1.006  $\mu$ g of 20:3n-6, respectively. Also,  
10 when no substrate is added, the level of 20:3n-6 is not detectable.

Once 20:3n-6 is generated by the M. alpina elongase (MAELO), the  $\Delta$ 5-desaturase can convert it to AA in the desired expression system. To test this hypothesis, the constructs pRAE-5 and pCGR-4 (a  $\Delta$ 5-desaturase containing  
15 plasmid) were co-transformed into S. cerevisiae 334 and screened for AA production. The substrate used was 25  $\mu$ M GLA (18:3n-6). If the M. alpina elongase (MAELO) is active in yeast, then the substrate will be converted to DGLA(20:3n-6), which the  $\Delta$ 5-desaturase will convert to  
20 AA(20:4n-6). The results in Figure 11 confirm the production of AA and therefore, the activity of the M. alpina elongase (MAELO).

The expression of  $\Delta$ 5-,  $\Delta$ 6-, and  $\Delta$ 12-desaturases, in yeast, along with the elongase, should result in the  
25 production of AA (see Figure 1) without the need for an exogenous supply of fatty acids.

#### Example IV

30 A Comparison of the Expression of M. alpina Elongase cDNA MAELO and S. cerevisiae Elongase ELO2 in Baker's Yeast

The ELO2 gene encoding for the yeast elongase was cloned from an S. cerevisiae genomic library (Origene, Rockville, MD) using the primers R0514 (5' -GGC TAT GGA TCC ATG AAT TCA CTC GTT ACT CAA TAT G-3') and R0515 (5' -CCT GCC AAG CTT TTA CCT TTT 5 TCT TCT GTG TTG AG-3') incorporating the restriction sites (underlined) *Bam*HI and *Hind*III (respectively). The ELO2 gene was cloned into the vector pYX242 at the *Bam*HI and *Hind*III sites, designated pRELO, transformed into the S. cerevisiae host 10 334 (Hoveland et al., supra) and screened for PUFA elongase activity. The vector plasmid was used as a negative control and 334(pRAE-5) was grown to compare the PUFA elongase activity. The cultures were grown as previously described with no galactose in the media and 25  $\mu$ M GLA added as a substrate. Figure 12 shows that amount of 20:3n-6 or DGLA produced 15 (elongated from 18:3n-6 or GLA) by 334(pRAE-5) was approximately 4 times the negative control containing the unaltered vector pYX242, while the two individual clones 334(pRELO-1) and 334(pRELO-2) were only twice the negative control. Additionally, when DGLA produced is expressed as a percent of 20 the total lipids (shown in parenthesis, Figure 12), the clones 334(pRELO-1) and 334 (pRELO-2) produced 0.153% and 0.2% DGLA respectively, while 334(pYX242) produced 0.185% DGLA. Hence all these strains produced comparable percentages of DGLA. The strain 334(pRAE-5), however, produced 0.279% DGLA, an increase 25 of 50.8% over 334(pYX242) (negative control). These data show that the S. cerevisiae elongase gene ELO2, even when overexpressed in yeast, does not elongate GLA to DGLA effectively. The M. alpina PUFA elongase activity is specific for this conversion as evidenced by the higher amount of DGLA 30 produced compared to the control, 334(pYX242).

Example VIdentification of Elongases from Other Sources Using MAELO

The TFastA algorithm (Pearson and Lipman, supra) is used to  
5 search for similarity between a query peptide sequence and the  
database DNA sequence translated in each of the six reading  
frames. Translated MAELO was used as the query for a TFastA  
search in GCG with the GenEMBL database (6/98) from GCG to  
identify other potential elongase sequences based on their amino  
10 acid similarity comparisons to translated MAELO. For example,  
in Figures 13 and 14, two alignments are shown between  
translations of two different C. elegans sequences from  
chromosome III and MAELO. C. elegans DNA sequence (GenBank  
accession # Z68749) was annotated denoting similarity with GNS1  
15 (ELO2), while the additional C. elegans DNA sequence (GenBank  
accession # U61954) was noted as similar to both GNS1 and SUR4  
(ELO3). These are spliced DNA fragments in which the introns  
have been removed from the genomic sequence, and the exons  
assembled and translated. The amount of amino acid identity  
20 between the putative PUFA elongases from C. elegans and  
translated MAELO are around 30%. This would point towards a  
common function in the fatty acid metabolism, e. g., a PUFA  
elongase. Figure 15 is another example of a translated C.  
elegans sequence (GenBank accession # AF003134) from chromosome  
25 III. The DNA sequence was identified that had DNA homology to  
the S. cerevisiae ELO2. Further inspection of this DNA sequence  
and its amino acid translation determined that there was  
homology to translated MAELO. C. elegans, therefore, may  
contain a PUFA elongase.

30 Figure 16 shows the alignments of translated DNA sequences  
from mouse and human, respectively, with translated MAELO. The

mouse sequence CIG30, GenBank accession # U97107, was isolated from brown adipose tissue and reported as being "similar to yeast SUR4 protein". As shown in Figure 16, amino acids numbered 130 to 152 in the U97107 translation contain a high degree of similarity to the translated MAELO. The human sequence, GenBank accession # AC004050, from chromosome 4 was from an HTGS (High Throughput Genome Sequence). There were no annotations contained with this sequence. However, translated AC004050 had 28.7% identity in 150 amino acids with translated MAELO. This gene fragment could be a fragment of a human PUFA elongase based on its amino acid similarity to translated MAELO.

Figure 17 shows the amino acid alignment of translated MAELO and a mammalian sequence (GenBank accession # I05465, PCT# WO 88/ 07577) which claims that the protein derived from expression of this sequence is a glycosylation inhibition factor. The amino acid identities between the two proteins, signifying that there could be related function, such as PUFA elongase activity.

These examples of other translated DNA sequences and their homology to the translated MAELO illustrate that any of the above examples could potentially be a PUFA elongase. These examples are not inclusive of all the possible elongases. However, use of MAELO or its amino acid translation as a query for database searches can identify other genes which have PUFA elongase activities.

Example VI

M. alpina cDNA Library Screening Using A Plaque Hybridization Method

In an effort to isolate additional PUFA elongase genes from M. alpina, a conventional plaque hybridization method was used to screen an M. alpina cDNA library made in a lambda vector. The DNA probe was generated based on MAELO nucleotide sequence 5 and was used to screen the M7+8 M. alpina cDNA library made in a λZiplox vector (Knutzon et al., J. Biol. Chem. 273:29360-29366 (1998)).

To make the DNA probe for screening the library, the MAELO cDNA was digested with *Nsp*I and *Pvu*I restriction endonucleases. 10 Three small DNA fragments, with an average size of approximately 300 bp, were produced and used as probes. The rationale for using a mixture of fragmented MAELO cDNA was based on the assumption that there might be a common region or domain in the amino acid sequence which is conserved among various PUFA 15 elongases present in M. alpina. Using MAELO DNA probes, the cDNA library was screened by a plaque hybridization technique according to standard protocol (Sambrook et al., Molecular Cloning, 2<sup>nd</sup> Ed., Cold Spring Harbor, 1989).

Briefly, 50,000 primary clones were plated and transferred 20 to nylon membranes. The membranes were denatured and hybridized with alpha <sup>32</sup>P-dCTP-labelled MAELO DNA probes overnight in the hybridization buffer which contained 20% formamide, 0.2% PVP, BSA, Ficoll, 0.1% SDS and 0.5 M NaCl. The filters were washed with 0.5X SSC at 37 °C and exposed to X-ray film for 25 autoradiography. This procedure was repeated three times. Four clones (designated as F1, F2, F3, and F4) which hybridized repeatedly were picked and suspended in SM buffer (Sambrook et al., supra) containing 7% DMSO.

The largest open reading frame of each candidate was 30 subcloned into yeast expression vector pYX242 (Novagen, Inc., Madison, Wisconsin). The cDNA clones F1 and F3 were subcloned

into pYX242 at the EcoRI site while F2 and F4 were subcloned at NcoI/HindIII sites. The recombinant pYX242 containing each candidate was transformed into SC334 (Hoveland et al., supra) for expression in yeast. To determine the elongase activity, as well as substrate specificity, SC334 containing each cDNA clone was grown in minimal media lacking leucine in the presence of 25 µM of GLA substrate as described in Example III. The fatty acid analysis was performed as described in Knutzon et al. (J. Biol. Chem. 273:29360-29366 (1998)). The results indicated that none of these four cDNA clones showed any significant activity in converting GLA to DGLA. Thus, the hybridization approach appeared to be unsuccessful in identifying additional PUFA elongases.

15

#### Example VII

##### Construction of Direct cDNA Expression Library of *M. alpina* in Yeast

To identify PUFA elongase genes other than MAELO, a different approach was taken to screen the *M. alpina* cDNA library. In particular, since Baker's yeast is incapable of producing long chain PUFAs due to the absence of respective desaturases and elongases, an attempt was made to construct an expression cDNA library of *M. alpina* in *Saccharomyces cerevisiae*. The vector pYES2 (Novagen, Inc., Madison, Wisconsin), containing the GAL1 promoter, was chosen for the expression of cDNA library in *S. cerevisiae*.

The conventional way by which a cDNA library is made (i.e. transformation of cDNA/vector ligated DNA mixture into host cells) is difficult in yeast because the transformation efficiency by direct electroporation of ligated DNA mix is very

low compared to the efficiency of purified supercoiled plasmid DNA. However, the major advantage of this method is to avoid amplification of primary clones which happens when the library is made in E. coli as an intermediate. Due to the limitation in 5 the number of colonies to be screened, it was decided to first optimize the efficiency of transformation in different S. cerevisiae strains using cDNA/vector ligated mix. The best results were obtained with a yield of  $4-5 \times 10^5$  transformants per  $\mu\text{g}$  of ligated DNA in S. cerevisiae strain SC334 (Hoveland et al., 10 supra).

To make a direct M. alpina cDNA expression library in yeast total RNA was isolated from the fungus. M. alpina fungus (ATCC # 32221) was plated onto cornmeal agar (Difco Laboratories, Detroit, MI) and grown at room temperature for 3-4 days. Once 15 fungus growth was visible, it was inoculated into 50 ml of potato dextrose broth and shaken at room temperature very slowly to formulate spores. Once spores were visible, the 50 ml culture was inoculated into a 1 liter culture of potato dextrose, and spores were grown for 72 hours. After filtering 20 through sterile gauze, the cells were immediately frozen into liquid nitrogen for future RNA extraction. Total RNA was prepared from 36 g of cell pellet using the hot phenol/LiCl extraction method (Sambrook et al., supra). The cell pellets were homogenized in a 10 mM EDTA, 1% SDS and 200 mM sodium 25 acetate, pH 4.8 solution. Phenol and chloroform were added to the homogenates, and the aqueous layer was extracted. The aqueous layer was back extracted one more time with phenol and chloroform. Then an equal volume of 4 M lithium chloride was added. The samples were ethanol precipitated on ice for 3 30 hours, and pellets were obtained by centrifugation. The RNA pellets were washed with 70% ethanol and resuspended in DEPC

treated water. Total RNA was quantitated by spectrophotometry and visualized by agarose gel electrophoresis to confirm the presence of 28S and 18S ribosomal bands. Approximately, 15 mg of total RNA were obtained from 36 gram of cell pellet.

5       The library was constructed according to the standard protocol (Sambrook et. al., Molecular Cloning, 2<sup>nd</sup> Ed.. Cold Spring Harbor, 1989). Messenger RNA was prepared from the total RNA using oligo dT cellulose affinity purification. Messenger RNA was reverse transcribed with oligo dT primer containing a  
10 *XhoI* restriction site using AMV reverse transcriptase. Following first strand cDNA synthesis, the second strand of cDNA was synthesized by adding E. coli DNA polymerase, E. coli DNA ligase and RNase H.

The *EcoRI* adaptor was ligated into the blunt-ended cDNA by  
15 T4 DNA ligase. The cDNA sample was kinased using T4 polynucleotide kinase and digested with *XhoI*, diluted with column buffer and passed through a Sephadryl S-400 column. The DNA samples were eluted by high salt buffer. Samples containing DNA from 400-5,000 bps were pooled and used for ligation into a  
20 pYES2 vector (Invitrogen Corp., Carlsbad, CA). The cDNA was ligated into the *EcoRI*/*XhoI* digested pYES2 vector using T4 DNA ligase. A large scale ligation reaction was carried out since a large amount of the ligated DNA (2-3 µg) is required in direct transformation of yeast.

25       To transform yeast cells directly with the cDNA/pYES2 ligated mixture, competent SC334 cells were prepared using the LiAc TRAFO method (Gietz et. al., Mol. Cell. Biol., 5: 255-269, 1995). Briefly, fresh culture of SC334 from the plate was inoculated into 50 ml YPD medium. The culture was grown at 30 °C  
30 with shaking until the OD at 600 had reached 1.0. Thirty ml of this starter was inoculated into 300 ml of YPD liquid medium and

incubated with shaking until the cell number of the culture reached ~  $3-5 \times 10^6$  cell/ml (approximately 3-4 h). The cells were harvested and washed with sterile water. The entire cell pellet was resuspended in 1.5 ml of freshly prepared 1X TE/LiAc (0.1M LiAc). These cells were used immediately for the transformations.

Seven hundred and fifty microliters of competent SC334 cells were aliquoted into 15 ml falcon tubes. Approximately 2 ug of cDNA/pYES2 ligated DNA were added to the cells along with carrier DNA and mixed gently. Three milliliters of sterile 40% PEG/LiAc was added to the cells and mixed gently but thoroughly. The cells were incubated at 30 °C for 30 min with shaking and subsequently given heat shock at 42 °C for 15 min. The cells were cooled, pelleted, and resuspended in 5 ml of 1X TE. A 100 ul aliquot of the above cells was plated onto fifty 150 mm selective agar plates lacking uracil (Ausubel et al., supra) and incubated at 30 °C for 3 days. A total of  $8 \times 10^5$  primary clones were obtained. Five colonies were pooled in 1 ml minimal media lacking uracil (Ausubel et al., supra) and glycerol added to prepare stocks. A total of 5,000 pools were made for screening.

Example VIII

MAD (M. alpina Direct) Screening in Yeast

The quality of the library was analyzed by determining the average size of the cDNAs in the library. Since the screening of the library was based on the expression of the cDNA, it was important to determine the average size of the cDNA present in the library. The expression library containing the longest cDNAs would be the best appropriate choice to isolate full-length cDNAs of interest. To this end, randomly selected pools

were plated onto selective agar plates, as described in Example VII, to obtain individual colonies. Forty different yeast colonies were randomly picked, and each colony was inoculated into 5 ml of selective liquid medium lacking uracil (as 5 described in Example VII) and grown, while shaking, for 24 hours at 30 °C. Plasmid DNA was extracted from these colonies by the bead beating method (Hoffman et al., Gene 57:267 (1987)) adapted as follows:

Pellets from 5 ml of culture were lysed in 0.5 ml of a 100 10 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA and 0.1% SDS solution. Sterile 0.5 mm glass beads of equal volume were added and manually vortexed for 3 minutes. Two hundred microliters of the same buffer were added, and the mixture was vortexed for an additional minute. The samples were centrifuged on high for 2 15 minutes, and cytoplasmic extract was then transferred to a fresh tube. An equal volume of phenol/CHCl<sub>3</sub> was added to the sample, vortexed and centrifuged again for 2 minutes. The aqueous layer was re-extracted twice and precipitated with 0.3 M sodium acetate and approximately 2.5 volumes of ethanol for 30 minutes 20 at -20 °C. The precipitates were washed with 70% ethanol and resuspended in water. To eliminate RNA and any protein contamination, the plasmid DNAs isolated from 40 different samples were further purified using the QIAprep Spin Miniprep Kit according to the manufacturer's protocol (Qiagen Inc., 25 Valencia, CA). The plasmid DNA samples were then restricted with EcoRI and XhoI restriction endonucleases to release the cDNA fragment, and the digest was analyzed on 1% agarose gel. The results indicated that the majority of the cDNAs of the direct library varied in length from 0.8 Kb to 1.5 Kb.

30 To screen the library, the glycerol stocks were thawed and approximately 0.5 ml was added to 5 ml of liquid selective media

lacking uracil (Ausubel et al., supra) and grown at 30°C for 24 hours. The culture was then transferred into 50 ml of liquid selective medium lacking uracil with 2% galactose and 25 µM GLA (substrate for the elongase enzyme) for 24 hours at 25 °C with 5 shaking. The GC-FAME analysis of the lipid content in the cell pellet of each induced culture was performed as previously described (Knutzon et al., supra). The MAELO (pRAE-5 in pYX242 grown in selective media lacking leucine) was used as a positive control in each batch run. MAELO had consistently been able to 10 convert 1.5% of GLA to DGLA (see Example III).

#### Example IX

##### Identification of a cDNA Encoding a Potential PUFA Elongase

15 After screening and analyzing approximately 750 individual pools by GC-FAME analysis, as described in Example VIII, one pool of five colonies (i.e., MAD708) appeared to have significant enzymatic activity in converting GLA to DGLA. This 20 activity was found to be approximately 5 fold higher than the M. alpina elongase activity (MAELO) in terms of DGLA/GLA ratio (Figure 19). This pool was tested again under identical assay conditions to confirm the initial findings. The repeat experiment showed 9.5% conversion of GLA to DGLA and was again 25 around 5 fold higher than M. alpina elongase activity (MAELO). These results strongly indicated that the MAD708 pool contained an elongase candidate which was specific for GLA as substrate. Since MAD708 was a pool of five different clones, it was necessary to isolate the individual cDNA clone which encoded for 30 elongase activity from this pool. To do this, the original MAD708 glycerol stock was plated onto a selective media agar

plate lacking uracil (Ausubel et al., supra). Thirty individual colonies were picked and grown in liquid selective medium, lacking uracil with 2% galactose, as previously described in Example VIII, in the presence of GLA. The cell pellet obtained 5 from each culture was then subjected to fatty GC-FAME analysis (Knutzon et al., supra) along with a positive control of 334 (pRAE-5) (MAELO in pYX242). The fatty acid analysis from the 30 individual clones from the MAD708 expression pool in yeast revealed that 5 of the 30 clones showed elongase activity in 10 converting GLA to DGLA. The fatty acid profiles of the active clones MAD708-2, MAD708-10, MAD708-18, MAD708-19 and MAD708-30 are shown in Figure 20. As shown in this Figure, MAD708-2, 10, and 30 produced the most DGLA, approximately 25 fold more than MAELO (pRAE-5). These 3 converted in the range of 41% to 49% of 15 GLA to DGLA. Other clones, MAD708-18 and MAD708-19, converted 8% and 21% of GLA to DGLA, respectively. All MAD708 clones converted a higher percentage of GLA to DGLA with respect to MAELO encoded elongase (3.4%).

20

Example XCharacterization of cDNAs Encoding Elongase

Plasmid DNA was extracted from SC334 yeast clones (MAD708 25 pool) that showed significant GLA specific elongase activity by the bead beating method, as described in Example VIII. To determine the size of the cDNA insert, PCR was performed using each plasmid DNA obtained from positive elongase clones as a template. The forward primer R0541 (5'- GAC TAC TAG CAG CTG TAA 30 TAC -3') and the reverse primer R0540 (5'- GTG AAT GTA AGC GTG ACA TAA -3') are in the multicloning site of the pYES2 vector

and were used to amplify the cDNA insert within the *EcoRI* and *XhoI* sites. PCR reaction was performed in a 50  $\mu$ l volume containing 4  $\mu$ l of plasmid DNA, 50 pmole of each primer, 5  $\mu$ l of 10X buffer, 1  $\mu$ l 10  $\mu$ M PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN) and 0.5  $\mu$ l of High Five Taq polymerase (Boehringer Mannheim, Indianapolis, IN). The amplification was carried out as follows: 2 mins. denaturation at 94 °C, then 94 °C for 1 min, 55 °C for 2 mins., and 72 °C for 3 mins. for 30 cycles, and 7 mins. extension at 72 °C at the end of the amplification. Analysis of PCR amplified products on a 1% agarose gel showed the sizes of the elongase cDNAs to be around 1.0 - 1.2 Kb. The plasmid DNAs, containing the potential elongase cDNAs, were designated as pRPB2, pRPB10, pRPB18, pRPB19, and pRPB30. Since the cDNA library was made in the pYES2 vector at the *EcoRI* and *XhoI* sites, the size of the cDNA present in each plasmid was further confirmed by digesting the above plasmids with *EcoRI* and *XhoI*.

The plasmid DNAs isolated from yeast were re-amplified in *E. coli* for long-term storage of the cDNA clones as well as for DNA sequencing. *E. coli* TOP10 (Invitrogen Corp., Carlsbad, CA) cells were transformed with the pRPB recombinant plasmids according to the manufacturer's protocol. The transformants obtained from each plasmid DNA were inoculated into LB containing ampicillin (50  $\mu$ g/ml) and grown overnight at 37 °C with shaking. Plasmid DNAs were isolated from these cultures by using QIAprep Spin Miniprep (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. The purified plasmid DNAs were then used for sequencing from both 5' and 3' ends. The DNA sequencing was performed by using a 373A Stretch ABI automated DNA sequencer (Perkin Elmer, Foster City, CA) according to the manufacturer's protocol. Primers used for

sequencing were the forward primer R0541 (5'- GAT TAC TAG CAG CTG TAA TAC -3') and the reverse primer R0540 (5'- GTG AAT GTA AGC GTG ACA TAA -3') contained in the multicloning sites of the pYES2 vector. The obtained nucleotide sequences were  
5 transferred to Sequencher software program (Gene Codes Corporation, Ann Arbor, MI) for analysis. The DNA sequence analysis revealed that all five elongase cDNAs contained the identical nucleotide sequence with a common overlap of 301 nucleotides. Each DNA sequence contains a putative start site  
10 at the beginning of the 5' end and a stop codon with poly A tail at the end of the 3' site. To further confirm the DNA sequence, internal forward primers R0728 (5'- GAG ACT TTG AGC GGT TCG -3') and R0730 (5'- TCT CTG CTG CGT TGA ACT CG -3'), along with reverse primers R0729 (5'- AAA GCT CTT GAC CTC GAA C  
15 -3') and R0731 (5'- AAC TTG ATG AAC GAC ACG TG -3') were designed within the cDNA, and used for sequencing of pRPB2, since this candidate possessed the highest elongase activity. The entire nucleotide sequence was analyzed by the Sequencher program (Figure 21), and the longest open reading frame deduced  
20 from the entire cDNA sequence in pRPB2 appeared to be 957 bp in length (Figure 22). The deduced open reading frame was then translated into the corresponding amino acid sequence, and the predicted sequence is shown in Figure 23. The elongase encoded by the cDNA (pRPB2) identified from M. alpina appears to be a  
25 318 amino acid long protein which is nearly identical in size with translated MAELO. This new elongase cDNA was designated as "GLELO" and its encoded protein has been named "GLA elongase".

Plasmid DNA pRPB2 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 on July 22, 1999 under the terms of the Budapest Treaty. It was accorded ATCC Deposit # PTA-402.

Example XIBiochemical Characterization of GLA Elongase (GLELO)A. Confirmation of GLA Elongase Activity

5 To further confirm the activity of the GLA elongase encoded by the pRPB2 recombinant plasmid, elongase activity screening was repeated on the yeast clone SC334 containing pRPB2 plasmid. This experiment was also conducted to assure consistent lipid extraction and to detect the activity of GLA elongase by  
10 averaging four independent experiments. The S. cerevisiae 334 glycerol stock containing pRPB2 was plated onto minimal media agar plates lacking uracil. Individual colonies were randomly picked and grown in minimal medium lacking uracil, as described in Example VIII. The four independent cultures were combined,  
15 and a 5 ml aliquot was used as an inoculum for four separate 50 ml cultures. The cultures were then grown in the presence of GLA and were subjected to fatty acid analysis along with a negative control of S. cerevisiae 334 containing pYES2, as described in Example VIII. The average elongase activity from  
20 four independent cultures of 334(pRPB2) with 25  $\mu$ M GLA is shown in Figure 24. The GLA elongase activity of each of the four independent samples of 334(pRPB2) appeared to be consistent with an average conversion of 62% GLA to DGLA.

25 B. Determination of GLELO Substrate Specificity for GLA Elongase

To analyze the substrate specificity of the GLA elongase, the culture of 334(pRPB2) was tested with different fatty acid substrates besides GLA (e.g., SA(18:0), OA(18:1), LA(18:2n-6), AA(20:4n-6), ADA(22:4n-6), ALA(18:3n-3), STA(18:4n-3), and  
30 EPA(20:5n-3)). Under identical assay conditions, the only other substrate utilized by the elongase enzyme was STA, a fatty acid

from the n-3 pathway. GLA elongase was able to convert 73% of STA to 20:4n-3 (Figure 25). From these experiments, it can be concluded that the GLA elongase has substrate specificity for both GLA and STA, indicating that it possesses elongase activity 5 along both the n-6 and n-3 pathways.

C. Co-expression of Fungal GLELO and Δ5-Desaturase Gene in Yeast

Once DGLA (20:3n-6) is produced by the GLA elongase, the Δ5-desaturase can convert it to AA (20:4n-6) in a desired co-expression system. This scheme, as depicted in Figure 1, can be tested by co-transforming *S. cerevisiae* 334 with plasmids pRPB2 and pRPE31 (the recombinant plasmid pYX242 containing a Δ5-desaturase cDNA (Figure 18) cloned at the EcoRI site. The co-transformed yeast cultures were supplemented with 25μM GLA and analyzed for AA synthesis. If both elongase and Δ5-desaturase enzymes are expressed, the GLA substrate will be converted to DGLA, which will then be converted to AA. The results in Figure 26A indicate that the sequential action of GLA elongase and Δ5-desaturase on GLA substrate resulted in an average conversion of 20 27% GLA to AA. Therefore, the GLA elongase has the ability to work with other enzymes in the n-6 PUFA synthetic pathway to produce desirable fatty acids.

To determine whether the above conversion is also true in n-3 pathways, the similar co-expression experiments were carried 25 out in the presence of 25 μM STA. Again, if both enzymes are expressed, the STA substrate will be converted to 20:4n-3 which will then be converted to EPA (20:5n-3) by the Δ5-desaturase. Figure 26B shows the results in which the production of EPA (approx. 40%) is observed. Once again, the GLA elongase

demonstrates its ability to work with Δ5-desaturase in the n-3 pathway to produce desirable fatty acids.

Example XII

5      Sequence Comparison Between GLELO and Other Fungal Elongases

The sequence analysis package of GCG (see Example I) was used to compare the GLELO sequence with known protein sequences. The nucleotide sequence of GLELO open reading frame was first 10 translated into amino acid sequence that was used as a query sequence to search Swissprot database (see Example I) using the FastA algorithm (see Example I). Based on amino acid sequence similarity, the best matches were found with S. cerevisiae YJT6 (an EST with unknown annotation) with 33.9% identity in 189 15 amino acid overlap, S. cerevisiae ELO2 (GNS1) with 25.8% identity in 295 amino acid overlap, and S. cerevisiae ELO3 (SUR4) with 25.2% identity in 313 amino acid overlap. The FastA alignment of GLELO with MAELO showed 30.9% identity in 275 amino acids (Figure 27). GCG Pileup program creates a multiple 20 sequence alignment from a group of related sequences using progressive, pairwise alignments (see Example I), and was used with the elongases described above. The Pileup results indicate that there are many conserved regions among the elongases including a putative histidine box, which is underlined (Knutzon 25 et. al., J. Biol. Chem. 273: 29360-29366, 1998) (Figure 28). Thus, although GLELO has similarity with MAELO, the difference in their encoded elongases may presumably be due to their substrate preference. GLA elongase can convert a higher percentage of GLA to DGLA than M. alpina elongase. In addition, 30 MAELO expression in S. cerevisiae showed elongation of saturated

and monounsaturated fatty acids in addition to GLA elongation to DGLA (see Example III).

5

Example XIIIIdentification of M. alpina MAELO Homologues in Mammals

The MAELO translated sequence was used to search the Unified Human Transcript Database of Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, Illinois 60064. This database was searched using Basic Local Alignment Search Tool (BLAST) (Altschul et al., Nuc. Acids Res. 25:3389-3402 (1997)) which "is a set of similarity search programs designed to explore all of the available sequence databases regardless of whether the query is a protein or DNA." Specifically, the tblastn algorithm was used (i.e., a protein query search to a nucleotide database translated in six reading frames). The contig (CC) sequences in the Unified Human Transcript Database are consensus sequences representing groups of expressed sequence tags (EST) cDNAs derived from the public domain and from the Incyte LIFESEQ™ database of ESTs (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304) that are clustered together on the basis of defined sequence homology, and assembled on the basis of sequence overlap. Two sequences from this database, CC067284R1 and CC1484548T1 had 28% identity in 242 amino acid overlap and 28.6% identity in 266 amino acid overlap, respectively, with the translated MAELO sequence. The two derived and edited sequences were designated as hs1 and hs2, respectively, and copied into the sequence analysis software package of GCG (see Example I). The translated MAELO sequence was aligned with translated HS1 (28.5% identity in 242 amino

acids) and HS2 (28.2% identity in 266 amino acids) cDNA sequences using the FastA algorithm, as shown in Figures 29 and 30, respectively. HS1 cDNA nucleotide sequence also had 86.9% identity in 844 bp with the I05465 nucleotide sequence (see 5 Example V). The translated HS2 cDNA sequence had 100% identity with the amino acid sequence from GenBank with accession number W74824 (see published PCT application WO9839448).

The National Center for Biotechnology Information (NCBI at <http://www.ncbi.nlm.nih.gov/>) was used to conduct database 10 searches using tblastn with the 28 amino acid sequence (DTIFIILRKQKL~~I~~FLHWYHHITVLLYSW) translated from AC004050 (a human sequence identified in a TFastA search, see Example V). This amino acid sequence contains a histidine box (underlined), which has a noted motif of desaturases (Knutzon et al., supra), and 15 both PUFA elongases, MAELO and GLELO (see Figure 28). A translated mouse sequence shown previously in Example V (GenBank Accession #U97107) and a translated C. elegans sequence (GenBank Accession # U41011) had the highest matches with this 28 amino acid query. The NCBI mouse EST database was searched again with 20 tblastn, using translated U41011 as a query. An additional mouse sequence was identified (GenBank Accession #AF014033.1), annotated as "putative involvement in fatty acid elongation." Three longer sequences (GenBank Accession #'s AA591034, AA189549, and AA839346) were identified through a tblastn search 25 of the mouse EST database with translated AF014033.1 and combined into one sequence designated as mm2. The FastA alignment (see Example I) of translated mm2 and MAELO is shown in Figure 31. Another related, but not identical mouse sequence (GenBank Accession #AI225632), was also identified in a tblastn 30 search of the mouse EST database with AF014033.1. The FastA alignment with translated AI225632 to MAELO is shown in Figure

32. The percent identity for both translated MM2 and AI 225632 with translated MAELO is 30.4% in 191 and 115 amino acid overlap, respectively. The level of amino acid identity with translated MAELO with these two translated mouse sequences  
5 identifies them as putative homologues of PUFA elongases.

Example XIV

Identification of M. alpina GLELO Homologues in Mammals

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The TFASTA algorithm, which compares a protein sequence to the database DNA sequence translated in each of the six reading frames, was used with translated GLELO as the query. The GenEMBL database from GCG was used to identify other potential 15 elongase sequences based on their amino acid similarity to translated GLELO. Three human sequences were found to have matches with the GLELO amino acid sequence. These sequences have GenBank accession numbers 1) AI815960, 2) AL034374, and 3) AC004050. AI815960, a Homo sapien EST sequence, has 40.3% 20 identity in 144 amino acid overlap with translated GLELO (see Figure 33). A translated region of the human genomic sequence AL034374, derived from chromosome VI has 46.7% identity in a 60 amino acid overlap with translated GLELO. This homologous region in AL034374 appeared to be a part of the HS1 amino acid 25 sequence which was shown to have homology with translated MAELO (see Example XIII). Therefore, HS1 sequence has similarity with both MAELO (see Figure 29) as well as GLELO (see Figure 34). A translated region of a human genomic sequence AC004050 from chromosome IV has 34.8% identity in 89 amino acid overlap with 30 translated GLELO (see Figure 35). The amino acid identities between GLELO and these human sequences indicate that the

proteins derived from these human sequences could have related function, such as PUFA elongase activity.

To identify a mouse cDNA similar to GLELO, TFastA searches were performed with the GenEMBL database using translated GLELO 5 as a query. From the TFastA searches, the three mouse sequences with the highest matches to translated GLELO were identified: (GenBank accession numbers 1) AF104033, 2) AI595258, and 3) U97107). AF104033 is annotated as "MUEL protein having putative fatty acid elongase with homology to yeast ELO3 (SUR4)" and is a 10 part of the sequence of MM2. The MM2 sequence was initially derived from AF104033 mouse sequence, but the entire MM2 sequence was finally obtained through further mouse EST database searches and also shown to have homology with translated MAELO (see Example XIII and Figure 31). When this MM2 amino acid 15 sequence was aligned with translated GLELO sequence using FastA, a 34.6% identity in 211 amino acid overlap was found (see Figure 36) indicating that MM2 also has homology with GLELO. AI595258 is a mouse cDNA clone having 5' similarity with yeast ELO3 elongase and is part of mouse EST cDNA AI225632. The AI225632 20 mouse sequence, which is a longer sequence than AI595258, was shown to have similarity with translated MAELO (see Figure 32). The AI225632 was also aligned with the translated GLELO, and the FastA alignment is shown in Figure 37. A 35.3% identity in 199 amino acid overlap has been found. The third sequence, U97107, 25 a mouse sequence, was annotated as "similar to yeast ELO3 (SUR4) gene." The FastA alignment of translated GLELO with U97107 is shown in Figure 38 where a 23.7% identity in 279 amino acid overlap was found. Previously, a region of U97107 was also found to have a high degree of homology with MAELO based on a 30 FastA alignment (see Example V and Figure 16).

The above searches clearly indicate that the same human and mouse sequences were obtained by using either MAELO or GLELO as a query.

Example XV

5     Identification of *M. alpina* GLELO and MAELO Homologues in Other PUFA Producing Organisms

A)    *Caenorhabditis elegans*:

10       A putative amino acid sequence deduced from a chromosomal sequence of *C. elegans* (GenBank Accession # U41011) was able to identify a partial sequence contained in the mouse MM2 putative PUFA elongase which has amino acid similarity with both GLA elongase (GLELO) and *M. alpina* elongase (MAELO). It was  
15 therefore conceivable that *C. elegans* homologues of GLELO or MAELO might be present in the nematode database. The putative amino acid sequences derived from GLELO and MAELO sequences were used as queries independently to search the nematode databases.  
A BLAST search (see Example XIII) was performed on wormpep16  
20 (blastp compares an amino acid query sequence against a nucleotide sequence database) and wormpep 16cDNAs (tblastn) databases which are predicted proteins and cDNAs obtained from the *C. elegans* genome sequencing project or EST's and their corresponding cDNA sequences, respectively. These sequence data  
25 were produced by the *C. elegans* Sequencing group, carried out jointly by the Sanger Centre and Genome Sequencing Center, and can be obtained from <ftp://ftp.sanger.ac.uk/pub/databases/wormpep/>. At least seven putative *C. elegans* translated sequences were identified by their amino acid  
30 sequence homology to the translated amino acid sequence of both GLELO and MAELO. The GenBank Accession #'s of those genomic

sequences containing the deduced amino acids were identified as Z19154, U68749 (2 deduced proteins (F56H11.4 and F56H11.3 (wormpep Accession #'s)), U41011, U61954 (2 deduced proteins (F41H10.7 and F41H10.8 (wormpep Accession #'s)), and Z81058.

5 Those underlined were identified in a previous search using translated MAELO as query (see Example V). As an example, the FastA amino acid alignments of translated U68749 (F56H11.4) with translated GLELO and MAELO are shown in Figures 39 and 40. Translated U68749 (F56H11.4) has 25-30% identity with both M.

10 alpina elongase and GLA elongase in approximately a 200 amino acid overlap (see Figures 39 and 40). For all seven translated putative C. elegans cDNAs, the FastA alignments to translated GLELO was between 25-30% identity in a 200 amino acid overlap, while the identity was 26-34% in at least a 188 amino acid

15 overlap for translated MAELO. The alignment similarities indicate that either translated GLELO or MAELO can be used to identify potential genes from C. elegans with elongase activity.

B) Drosophila melanogaster:

20 The translated deduced cDNA from the genomic sequence U41011 (C. elegans) had its highest match with a Drosophila melanogaster EST, accession number AI134173 in a blastn search (compares a nucleotide query sequence against a nucleotide database) of the "other ESTs" database through NCBI (see Example XIII) and was assembled with an overlapping DNA EST fragment, accession number AI517255. The translated DNA fragment DM1, derived from the two overlapping sequences was aligned with translated GLELO as well as MAELO (see Figures 41 and 42) using FastA in GCG (see Example I). The alignments showed 27.2%

25 identity with GLA elongase in a 206 amino acid overlap and 30% identity with M. alpina elongase in a 237 amino acid overlap.

Thus, based on amino acid similarity, the DM1 could be a potential homologue to GLELO or MAELO having PUFA elongase-like activity. Moreover, using DNA sequences of GLELO and MAELO as queries for database searches, homologues with PUFA elongase 5 activity from Drosophila can be identified.

Example XVI

Cloning and Expression of A Human PUFA Elongase Homologue

10 Many potential PUFA elongase sequences were identified based on their amino acid similarities to translated GLELO and/or MAELO. To determine the potential elongase activities of these sequences, the cDNA encoding the full-length protein is then identified, cloned, and expressed, as demonstrated in the 15 present example.

Primers R0719 (5' -GGT TCT CCC ATG GAA CAT TTT GAT GCA TC- 3') and R0720 (5' -GGT TTC AAA GCT TTG ACT TCA ATC CTT CCG- 3') were designed based on the putative HS1 sequence, and used to amplify the human liver Marathon-Ready cDNA (Clontech 20 Laboratories, Inc., Palo Alto, California). The polymerase Chain Reaction (PCR) was carried out in a 50  $\mu$ l volume containing: 5  $\mu$ l of human liver Marathon-Ready cDNA, 50 pmole each primer, 1 $\mu$ l 10 mM PCR Nucleotide Mix (Boehringer Mannheim Corp., Indianapolis, IN), 5  $\mu$ l 10 X buffer and 1.0 U of Advantage 25 KlenTaq Polymerase Mix (Clontech Laboratories, Inc., Palo Alto, CA). Thermocycler conditions in Perkin Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins, and 72 °C for 3 mins. PCR was followed by an additional extension cycle at 72 °C for 7 minutes.

The PCR amplified product was run on a gel, an amplified fragment of approximately 960 bp was gel purified, the termini of the fragment filled-in with T4 DNA polymerase (Boehringer Mannheim, Corp., Indianapolis, IN), and cloned into pCR-Blunt 5 Vector (Invitrogen Corp., Carlsbad, CA) following manufacturer's protocol. The new plasmid was designated as pRAE-52, and the putative PUFA elongase cDNA in this clone was sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence in plasmid pRAE-52 is 10 shown in Figure 43, and the translated sequence is shown in Figure 44.

The putative PUFA elongase cDNA from plasmid pRAE-52 was then digested with *NcoI/HindIII*, gel purified, and ligated into pYX242 (*NcoI/HindIII*). The new plasmid was designated as pRAE-15 58-A1. (Plasmid 58-A1 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number PTA-566.)

The construct pRAE-58-A1 was transformed into S. cerevisiae 20 334 (Hoveland et al., supra) and screened for elongase activity. The negative control strain was S. cerevisiae 334 containing pYX242 vector. The cultures were grown for 24 hours at 30°C, in selective media (Ausubel et al., supra), in the presence of 25 µM of GLA or AA. In this study, DGLA or adrenic acid (ADA, 22:4n-25 6), respectively, was the predicted product of human elongase activity. When GLA was used as a substrate, the yeast cells containing the human elongase cDNA contained elevated levels of DGLA compared to control cells, 2.75% vs. 0.09% of total fatty acids, respectively (see Figure 45). When AA was used as a 30 substrate, the yeast cells containing the human elongase cDNA contained elevated levels of ADA compared to control cells, none

detected vs. 1.21% of total fatty acids, respectively. Thus, the human elongase converts both 18 and 20 carbon chain long PUFAs to their respective elongated fatty acids.

The yeast cells containing the human elongase cDNA also had 5 elevated levels of monounsaturated fatty acids including 18:ln-7, 20:ln-7, 20:ln-9, and 18:ln-5, compared to the control strain. Therefore, these results indicate that the identified human elongase is capable of utilizing PUFAs as well as monounsaturated fatty acids as substrates. Thus, this human 10 sequence HSEL01, and its encoded protein (HSEL01p), possess elongase activity independent of substrate specificity.

To further confirm the substrate specificity of the human elongation enzyme, described above and referred to herein as HSEL01, the recombinant yeast strain 334(pRAE-58-A1) was grown 15 in minimal media containing n-6 fatty acids GLA, AA, or n-3 fatty acids ALA, STA, or EPA. The lipid profiles of these yeast cultures, when examined by GC and GC-MS, indicated that there were accumulations of DGLA, ADA,  $\omega$ 3-eicosatrienoic acid (ETrA, C20:3n-3), ETA, and DPA, respectively (Figure 51). The levels 20 of these fatty acids were 7.29% (DGLA), 6.26% (ADA), 6.15% (ETrA), 10.06% (ETA), and 6.66% (DPA), respectively, of the total fatty acids in the strain containing the pRAE-58-A1 sequence. These represented 78.3%, 42.7%, 30.4%, 79.2%, and 25 71.7% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms.

The yeast cells expressing the recombinant HSEL01 sequence, compared to the control cells, also contained significantly elevated levels of C18:ln-7 and C20:ln-7, and to a lesser extent, eicosenoic acid (EA, C20:ln-9) (Figure 45). This 30 finding suggested that the recombinant HSEL01 protein (HSEL01p) might also be involved in the elongation of monounsaturated

fatty acids of 16 or 18 carbon lengths. To confirm this hypothesis, 25  $\mu$ M of exogenous OA was added as a substrate to the recombinant yeast strain 334(pRAE-58-A1). After incubation, the accumulation of EA at 2.25% of the total fatty acids  
5 demonstrated that the expressed HSELO1 enzyme could elongate monounsaturated fatty acids (Figure 51). However, the conversion of OA to EA by recombinant HSELO1p was only 8.9%; this conversion was significantly lower than the endogenous conversion of C16:1n-7 (to C18:1n-7) or C18:1n-7 (to C20:1n-7),  
10 which was 20.4% and 58.1%, respectively.

To determine whether the substrate concentration affects the conversion of 18 and 20 carbon fatty acids to the respective elongated products, two different concentrations of GLA, AA, and EPA were examined (Figure 52). When 25  $\mu$ M of the substrates GLA,  
15 AA, and EPA were added exogenously, the levels of the fatty acids produced by two carbon elongation were 3.95% (DGLA), 2.91% (ADA), and 4.82% (DPA), respectively, of the total fatty acids in the lysates of 334(pRAE-58-A1). These represented 62.4%, 27.5%, and 70.3% conversions of the substrate fatty acids,  
20 respectively, to the products elongated by two carbon atoms. When 100  $\mu$ M of the substrates GLA, AA, and EPA were added, the levels of the fatty acids produced by two carbon elongation were 9.56% (DGLA), 3.90% (ADA), and 11.50% (DPA), respectively, of the total fatty acids in the lysates of 334(pRAE-58-A1). These  
25 represented 39.8%, 15.7%, and 45.7% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. Although the addition of more substrates led to higher percentages of the two carbon elongated products, the overall conversion rate decreased by at least 35%.

To further confirm the substrate specificity of HSEL01p, the recombinant yeast strain 334 (pRAE-58-A1) was grown in minimal media containing 25  $\mu$ M of saturated, monounsaturated, or PUFAs. The lipid profiles of these various substrates revealed 5 that HSEL01p is not involved in the elongation of saturated fatty acids such as palmitic acid (PA, C16:0), stearic acid (SA, C18:0), arachidic acid (ARA, C20:0), behenic acid (BA, C22:0) (Figure 53A). HSEL01p is also not involved in the elongation of monounsaturated fatty acids OA and EA. When PTA was added as a 10 substrate, 12.76% of the total fatty acids was OA. However, this is not an increase in the level of OA compared to the samples where PTA was not added, as OA was 25-31% of the total fatty acids in all samples. HSEL01p is involved in the elongation of n-6 PUFAs LA, GLA, and AA, but not DGLA or ADA 15 (Figure 53B). The lipid profiles of these yeast cultures indicated that there were accumulations of C20:2n-6, DGLA, and ADA, respectively, but not C22:3n-6 or C24:4n-6. The levels of these fatty acids were 0.74% (C20:2n-6), 2.46% (DGLA), and 2.14% (ADA), respectively, of the total fatty acids in the lysates of 20 334 (pRAE-58-A1). These represented 13.2%, 51.4%, and 27.1% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. HSEL01p is also involved in the elongation of n-3 PUFAs ALA, STA, and EPA, but not DPA (Figure 53C). The lipid profiles of these yeast 25 cultures indicated that there were accumulations of ETrA, ETA, and DPA, respectively, but not C24:5n-3. The levels of these fatty acids were 1.03% ETrA, 2.24% (ETA), and 3.19% (DPA), respectively, of the total fatty acids in the strain containing the pRAE-58-A1 sequence. These represented 22.2%, 61.9%, and 30 39.5% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. All results

confirmed that the expression of HSEL01 from human liver in yeast resulted in the elongation of various long-chain PUFAs in n-6 and n-3 fatty acid pathways.

5

Example XVIICloning, Expression and Characterization of a C. elegans PUFA Elongase

Several putative C. elegans elongases were identified with 10 amino acid homology to both translated GLELO and MAELO. As with the human cDNA sequence, cloning of a cDNA and expression in yeast was used to determine if indeed it was a PUFA elongase.

Primers R0738 (5' -AAT CAG GAA TTC ATG GCT CAG CAT CCG CTC GTT CAA C -3') and R0739 (5' -CCG CTT GTC GAC TTA GTT GTT CTT CTT CTT TGG CAC -3') with restriction sites *Eco*RI and *Sall* (underlined), respectively, were based on the putative cDNA sequence contained in the genomic sequence U68749 (wormpep cDNA accession #F56H11.4.) A PCR amplification was performed in a 100  $\mu$ l volume containing: 250 ng excised C. elegans library cDNA 20 (OriGene Technologies Inc., Rockville, MD), 50 pmole each primer, 10  $\mu$ l 10X reaction buffer (Boehringer Mannheim Corp., Indianapolis, IN), 1  $\mu$ l 10 mM PCR Nucleotide mix (Boehringer Mannheim Corp., Indianapolis, IN), and 2.5 U Taq polymerase (Boehringer Mannheim Corp., Indianapolis, IN). Thermocycler 25 conditions in a Perkin Elmer 9600 (Norwalk, CT) were as follows: 95 °C for 5 mins, then 25 cycles of 94 °C for 30 secs, 55 °C for 2 mins, and 72 °C for 2 mins. PCR was followed by an additional cycle of 72 °C for 7 minutes.

The PCR amplified product was purified from an agarose gel, 30 cut with *Eco*RI and *Sall*, ligated to pYX242 (Invitrogen Corp., Carlsbad, CA) (linearized with *Eco*RI and *Sall*) using the Rapid

Ligation kit (Boehringer Mannheim Corp., Indianapolis, IN), according to the manufacturer's protocol and transformed into E. coli Top10 cells (Invitrogen Corp., Carlsbad, CA). The new plasmids, designated pRET-21 and pRET-22 (two individual clones from the ligation), were sequenced with the 373A Stretch DNA sequencer ABI (Perkin Elmer, Foster City, CA), and the cDNA sequences were identical. The 867 base cDNA nucleotide sequence of the plasmid pRET-22 containing the putative elongase is shown in Figure 46 and the translated sequence of 288 amino acids is shown in Figure 47. (Plasmid pRET-22 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on August 19, 1999, under the terms of the Budapest Treaty and was accorded deposit number PTA-565.)

The plasmids pRET-21 and -22 were transformed into S. cerevisiae 334 as previously described (see Example III) and the resulting yeast cultures (334(pRET-21) and 334(pRET-22)) grown in 100 ml of selective media without leucine (Ausubel et al, supra) for 48 hours at 20 °C in the presence of 50 µM GLA and AA. The cell pellets were collected and subjected to fatty acid analysis and the results shown in Figure 48. DGLA, the predicted product from GLA elongation, was found to be an average of 1.79% of the total lipid in the two samples, versus 0.13% for the negative control (334 containing plasmid pYX242) indicating that the enzyme encoded by both pRET-21 and pRET-22 possessed GLA elongase activity. The percent conversion of GLA to DGLA by 334(pRET-21) and 334(pRET-22) was 11.1% and 19.4% respectively with an average of 15.25%. Interestingly, almost no elongation of AA or any endogenous fatty acid was observed (Fig. 48). These results indicate that the elongase encoded by this newly identified C. elegans cDNA, CEELO1, is able to

specifically elongate GLA to DGLA, suggesting that it may be a *C. elegans* homologue of GLA elongase.

To further confirm the GLA elongation activity of CEELO1, the experiment described in the paragraphs above was repeated  
5 with the exception that GLA and AA were added to cultures of 334(pRET-22) separately. Again, GLA was elongated to DGLA with a 38.2% conversion rate. No elongation activity of AA was detected as shown in Figure 61. In this case, the percent conversion appears to be double that described in previous  
10 results (see Figure 48) and may either be due to the absence of the additional substrate (AA) or the subculturing of the yeast. CEELO1 has the additional activity of elongating endogenous 16:1n-7 to 18:1n-7 with a 9.12% conversion rate compared to 3.9% control culture 334(pYX242) under identical conditions. Thus,  
15 the *C. elegans* elongation enzyme possesses a major elongation activity for a C18 polyunsaturated fatty acid and a minor activity for a C16 monounsaturated fatty acid.

Additionally, to further determine the substrate specificity of CEELO1, 50  $\mu$ M of each substrate besides GLA (e.g.,  
20 SA (18:0), OA (18:1), LA (18:2n-6), DGLA (20:3n-g), AA (20:2n-6), ADA (22:4n-6), ALA (18:3n-3), PA (18:0), EPA (20:5n-3) and STA (18:4n-2)) was added individually to cultures of 334(pRET-22) and grown for 48 hours at 20 °C, as described in Example XVII. STA was the only exogenously added substrate that was  
25 elongated. The CEELO1 elongated 13% of STA incorporated to ETA (20:4n-3) (see Figure 62).

Parallel to Examples III and XI, the *C. elegans* CEELO1 gene in the plasmid pRET22 and *M. alpina* Δ5 desaturase (pCGR-4; see Example III) were co-expressed in yeast to determine if AA or  
30 EPA could be produced from exogenously added GLA or STA, respectively. When a yeast culture containing both pRET22 and

pCGR4 plasmids was grown in the presence of 50  $\mu$ M GLA or STA in media lacking leucine and uracil, the percent conversion to the final products of AA and EPA, respectively, appeared identical (27% conversion) (see Figure 63). Thus, simultaneous  
5 heterologous expression of CELO1 and a  $\Delta$ 5 desaturase results in the biosynthesis of AA and EPA from GLA and STA, respectively in yeast.

10

Example XVIIIIsolation of a Putative Human Elongase cDNA Based on AC004050 Sequence

To isolate the full length putative elongase cDNA based on  
15 the AC004050 sequence, primers RP735 (5' -CCT CCT GAA TTC CAQA  
CAC TAT TCA GCT TTC -3') and R073 (5' -TAA TAC GAC TCA CTA TAG  
GG -3') were used to PCR amplify the human liver Marathon-Ready  
cDNA (Clontech Laboratories, Inc., Palo Alto, CA). The PCR was  
carried out using the Advantage<sup>TM</sup> cDNA PCR Kit (Clontech  
20 Laboratories, Inc., Palo Alto, CA) with 5  $\mu$ l of human liver  
Marathon-Ready cDNA and 50 pmole each primer following  
manufacturer's instructions. Thermocycler conditions in Perkin  
Elmer 9600 (Norwalk, CT) were as follows: 94 °C for 2 mins, then  
30 cycles of 94 °C for 1 min., 58 °C for 2 mins., and 72 °C for  
25 3 mins. PCR was followed by an additional extension at 72 °C  
for 7 mins.

The PCR amplified product was run on a gel, an amplified  
fragment of approximately 1 Kb was gel purified, the termini of  
the fragment were filled in with T4DNA polymerase (Boehringer  
30 Mannheim, Corp., Carlsbad, CA) following manufacturer's  
instructions. The new plasmid was designated as pRAE-59, and

the putative PUFA elongase cDNA in this plasmid, designated as HS3, was sequenced using the ABI 373A Stretch Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongase cDNA sequence HS3 is shown in Figure 49, and the translated sequence 5 is shown in Figure 50.

Example XIX

Cloning and Expression of a Mouse PUFA Elongation Enzyme

10       The National Center for Biotechnology Information (NCBI at <http://www.ncbi.nlm.nih.gov>) was used to conduct database searches using blastn with the mouse EST sequence AI225632 (see Example XIII). Three mouse EST sequences were identified (GenBank Accession #'s AI428130, AI595258, and AA061089), and  
15       assembled to generate a putative full-length elongation enzyme sequence, designated as MELO4. Primers R0819 (5' -ATG ATG CCA TGG AGC AGC TGA AGG CCT TTG- 3') and R0820 (5' -CAG TCT CTG CTT TAA AAC AAG CTC GTC- 3') were designed based on the putative full length mouse elongation enzyme sequence, and used to  
20       amplify the mouse brain Marathon-Ready cDNA (Clontech Laboratories, Inc., Palo Alto, California). The Polymerase Chain Reaction (PCR) was carried out as previously described (Example XVI). The PCR amplified product was run on a gel, an amplified fragment of approximately 1,000 bp was gel purified,  
25       the termini of the fragment were digested with NcoI and DraI (Boehringer Mannheim, Corp., Indianapolis, IN), and the fragment was cloned into pYX242 (NcoI/HindIII). The new plasmid was designated as pRAE-84, and the putative PUFA elongation enzyme cDNA in this clone was sequenced using ABI 373A Stretch DNA  
30       Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA

elongation enzyme cDNA sequence in plasmid pRAE-84 is shown in Figure 54, and the translated sequence is shown in Figure 55.

The construct pRAE-84 was transformed into S. cerevisiae 334 (Hoveland et al., supra) and screened for elongase activity. 5 The negative control strain was S. cerevisiae 334 containing pYX242 vector. The cultures were grown for 42-48 hours at 30°C, in selective media (Ausubel et al., supra), in the presence of 25 µM of GLA, AA, ADA, STA, EPA, or DPA. The lipid profiles of these yeast cultures indicated that GLA was not elongated to the 10 expected product of DGLA. However, there were accumulations of ADA,  $\omega_6$ -tetracosatetraenoic acid (TTA, C24:4n-6), ETA, DPA, and  $\omega_3$ -tetracosapentaenoic acid (TPA, C24:5n-3), respectively (Figure 56). The n-6 fatty acid substrate AA was converted to ADA, which was subsequently converted to TTA, and the n-3 fatty 15 acid EPA was converted to DPA, which was subsequently converted to TPA. The levels of these fatty acids were 0.64% (ADA), 1.07% (TTA), 1.47% (DPA), and 7.06% (TPA), respectively, of the total fatty acids in the strain containing the pRAE-84 sequence. These represented 10.4%, 62.6%, 32.7%, and 82.8% conversions of 20 the substrate fatty acids, respectively, to the products elongated by two carbon atoms. The C22 substrates ADA and EPA were elongated to 2.4% (TTA) and 3.82% (TPA) of the total fatty acids. These represented 9.2% and 43.9% conversions of the substrate fatty acids, respectively. The expression of MELO4 in 25 yeast results in the conversion of C20 and C22 fatty acids to the respective elongated products. The conversion rate of C22 to C24 fatty acids is much greater when the exogenously added substrate is C20 fatty acid.

To further confirm the substrate specificity of MELO4 30 protein (MELO4), the recombinant yeast strain 334(pRAE-84) was

grown in minimal media containing 25  $\mu$ M of saturated, monounsaturated, or polyunsaturated fatty acids. The lipid profiles of these various substrates revealed that MELO4p is not involved in the elongation of saturated fatty acids such as PA, 5 SA, ARA, or BA (Figure 57A). MELO4p is also not involved in the elongation of monounsaturated fatty acids PTA, OA, or EA. MELO4p is involved in the elongation of n-6 PUFAs AA and ADA, but not LA or DGLA (Figure 57B). The lipid profiles of these yeast cultures indicated that there were accumulations of ADA 10 and TTA, but not C20:2n-6 or C22:3n-6. When AA was added exogenously, the levels of product fatty acids were 0.5% (ADA) and 0.39% (TTA), and when ADA was added exogenously, the level of product fatty acid was 1.3% (TTA) of the total fatty acids in the strain containing the pRAE-84 sequence. These represented 15 8.7%, 43.8%, and 7.3% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. MELO4p is also involved in the elongation of GLA to DGLA. The lipid profile of the strain containing the pRAE-84 sequence, in presence of GLA, had 0.43% of DGLA, which represented 14.7% 20 conversion of GLA to DGLA. MELO4p is also involved in the elongation of n-3 PUFAs EPA and DPA (Figure 53C). The lipid profiles of these yeast cultures indicated that there were accumulations of DPA and TPA. When EPA was added, the levels of these fatty acids were 1.21% (DPA) and 3.38% (TPA), and when DPA 25 was added, the level of the product fatty acid was 3.09% (TPA) of the total fatty acids in the strain containing the pRAE-84 sequence. These represented 24.0%, 73.6%, and 46.4% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms. MELO4p is also involved in the 30 elongation of STA to C22:4n-3. When STA was added, the levels of fatty acids produced by two-carbon elongation were 0.3% ETA

and 0.23% C22:4n-3. These represented 11.1% and 43.4% conversions of substrate fatty acids to the products elongated by two carbon atoms. MELO4p also appeared to be involved in the elongation of ALA; however, the small amount of the fatty acid 5 produced by two-carbon elongation (0.16% of ETrA) may not be significant. All results confirmed that the expression of MELO4 from mouse brain in yeast resulted in the elongation of C20 and C22 long-chain PUFAs in n-6 and n-3 fatty acid pathways.

10

Example XXIdentification, Cloning, and Expression of HSELO1 Homologue from Mouse

The National Center for Biotechnology Information (NCBI at 15 <http://www.ncbi.nlm.nih.gov>) was used to conduct database searches using blastn with the HSELO1 sequence. Two human EST sequences were identified (GenBank Accession #'s AI787925 and AI746838) and the respective cDNA clones (I.M.A.G.E. Consortium Clone ID's 2076831 and 206182) were purchased through Research 20 Genetics (Huntsville, AL). Primers R0833 (5' -GGT TTT ACC ATG GAA CAT TTC GAT GCG TCA C- 3') and R0832 (5' -CGA CCT GCA GCT CGA GCA CA- 3') were designed based on 5' sequence of the putative mouse elongation enzyme, and the cDNA clone vector, respectively. Primers R0833 and R0832 were used to amplify the 25 mouse cDNA clone 206182. The Polymerase Chain Reaction (PCR) was carried out as previously described (Example XVI). The termini of the PCR amplified product were filled-in with T4 DNA polymerase (Boehringer Mannheim, Corp., Indianapolis, IN) and the 5' region was digested with NcoI. The modified fragment was 30 run on a gel, an amplified fragment of approximately 2.4 Kp was gel purified, and the fragment was cloned into pYX242.

(NcoI/EcoRV). The new plasmid was designated as pRAE-87, and the putative PUFA elongation enzyme cDNA in this clone, MELO7, was sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongation enzyme 5 cDNA sequence in plasmid pRAE-87 (MELO7) is shown in Figure 58, and the translated sequence is shown in Figure 59.

The construct pRAE-87 was transformed into S. cerevisiae 334 (Hoveland et al., supra) and screened for elongase activity. The negative control strain was S. cerevisiae 334 containing 10 pYX242 vector. The cultures were grown for 42-48 hours at 30°C, in selective media (Ausubel et al., supra), in the presence of 25 M of GLA, AA, STA, EPA, DPA, or ADA. The lipid profiles of the yeast cultures expressing MELO7 indicated that there were accumulations of DGLA, ADA, and ETA, respectively (Figure 60). 15 The levels of these fatty acids were 4.1% (DGLA), 6.33% (ADA), 3.4% (ETA), and 6.18% (DPA), respectively, of the total fatty acids in the strain containing the pRAE-87 sequence. These represented 78.7%, 36.0%, 81.0%, and 57.4% conversions of the substrate fatty acids, respectively, to the products elongated 20 by two carbon atoms. MELO7 protein (MELO7) was not involved in the elongation of ADA. MELO7p also appeared to be involved in further elongation of the fatty acid DPA produced by two-carbon elongation to TPA when EPA was the added substrate, and when DPA was added. However, the small amounts of the product fatty 25 acids (0.27% and 0.25% of TPA) may not be significant. The yeast cells expressing the recombinant MELO7 sequence, compared to the control cells, also contained significantly elevated levels of C18:1n-7 and C20:1n-7. All results confirmed that the expression of MELO7 from mouse embryo in yeast resulted 30 in the elongation of various long-chain PUFAs in n-6 and n-3 fatty acid pathways, and that MELO7 was a homologue of HSEL01.

Example XXICloning and Expression of a Fungal PUFA Elongation Enzyme

5

The translated amino acid sequences of the four elongases, HSELO1, MELO4, GLELO, and CEELO, were aligned to identify areas of homology (Figure 64). A primer was designed based on a block of sequences that showed similarities (underlined). Primer 10 RO895 (5' -GTA GTA WGA GTA CAT GAT WAC GTG GAT RAA WGA GTT WAG- 3') and vector primer RO898 (5' -CCC AGT CAC GAC GTT GTA AAA CGA CGG CCA G- 3') were used to amplify the cDNA from *Thraustochytrium aureum* 7091 (T7091). PCR was carried out in a . 50  $\mu$ l volume containing: 1  $\mu$ l of T7091 cDNA, 0.2  $\mu$ M dNTP mix, 50 15 pM each primer, 5  $\mu$ l of 10 X buffer, 1.5  $\mu$ l of 50 mM MgSO<sub>4</sub>, and 0.5 U of Taq DNA Polymerase. Thermocycler conditions in Perkin 20 Elmer 9600 were as follows: 94°C for 3 min, then 30 cycles of 95°C for 45 sec., 55 °C for 30 sec., and 68°C for 2 min. The PCR amplified mixture was run on a gel, an amplified fragment of approximately 750 bp was gel purified, and the isolated fragment 25 was cloned into the pCR-blunt vector (Invitrogen, Co., Carlsbad, CA) .

Twelve clones were prepared and sequenced. All twelve 25 clones had the same sequence, and the consensus sequence was designated as cld6 (Figure 65). The translated amino acid sequence of cld6 (Figure 66) had 34.7% identity in 190 amino acids with HSELO1, 35.8% identity in 187 amino acids with MELO4, 45.6% identity in 160 amino acids with GLELO, and 32.9% identity in 155 amino acids with CEELO. A new primer was designed based 30 on the 5' sequence of cld6, at the first Met. RO1160 (5' -AAG GAA CCA TGG CAA ACA GCA GCG TGT GGG ATG- 3'), which has an added

NcoI site (underlined), and vector primer R0899 (5' -AGC GGA TAA CAA TTT CAC ACA GCA AAC AGC- 3') were used to amplify the T7091 cDNA. The Polymerase Chain Reaction (PCR) was carried out as described above. The PCR amplified product was run on a gel, an 5 amplified fragment of approximately 1.2 Kb was gel purified, the termini of the fragment were digested with NcoI and HindIII (Boehringer Mannheim, Corp., Indianapolis, IN), and the fragment was cloned into pYX242 (NcoI/HindIII). The new plasmids were designated as pRAT-4-A1, pRAT-4-A2, pRAT-4-A3, pRAT-4-A4, pRAT- 10 4-A6, pRAT-4-A7, and pRAT-4-D1. (Plasmid DNA pRAT-4-A7 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, on June 29, 2001, under the terms of the Budapest Treaty, and was accorded deposit number ATCC PTA-3490.) The putative PUFA elongation enzyme 15 cDNAs in these clones were sequenced using ABI 373A Stretch DNA Sequencer (Perkin Elmer, Foster City, CA). The putative PUFA elongation enzyme cDNA sequences in plasmids pRAT-4-A1 through pRAT-4-D1 are shown in Figures 67-73, and the translated sequences are shown in Figures 74-80, respectively.

20 The constructs pRAT-4-A1 through pRAT-4-D1 were transformed into S. cerevisiae 334 (Hoveland et al., *supra*) and screened for elongase activity. S. cerevisiae 334 containing the unaltered pYX242 vector was used as a negative control. The cultures were grown for 48 hours at 24°C, in selective media (Ausubel et al., 25 *supra*), in the presence of 25 µM of GLA or EPA. In this study, DGLA or ω3-docosapentaenoic acid (DPA, 22:5n-3), respectively, was the predicted product of the elongase activity. The lipid profiles of these yeast cultures indicated that GLA was elongated to DGLA in all the samples, except for 334(pRAT-4-A3) 30 (Figure 81). This was expected in that the cDNA sequence of

pRAT-4-A3 had several stops at the 5' end of the sequence, which would result in a truncated version of the translated enzyme (Figure 76). Although the DGLA level was very low for 334(pRAT-4-A2), the GLA level was the lowest in this sample; therefore, 5 the conversion level of the fatty acid to its elongated form was significant at 28.4%. What was not expected was that there was definite substrate specificity among the expressed enzymes. The cultures of 334(pRAT-4-A1) and 334(pRAT-4-A2) had very low levels of DPA when EPA was added as a substrate, indicating that 10 the expressed enzymes in these cultures preferred the elongation of the 18-carbon chain long PUFA, and not the 20-chain long PUFA, EPA. The cultures of 334(pRAT-4-A4), 334(pRAT-4-A6), 334(pRAT-4-A7), and 334(pRAT-D1) all had significant levels of DPA present, indicating that the expressed enzymes in these 15 cultures were involved in the elongation of both 18- and 20-carbon chain long PUFAs to their respective elongated fatty acids.

The amino acid sequences of the 6 active clones were compared to determine if the substrate preferences were dictated 20 by the translated sequences (Figure 82). The cDNA sequences of pRAT-4-A1 and pRAT-4-A2 differed from other sequences in that they had a mutation at Y377C and V371A, respectively. This must be a critical region of the enzyme for 20-carbon chain elongation. The other sequences also had mutations, pRAT-4-A4 (I475V), pRAT-4-A6 (D26G and V458A), and pRAT-4-D1 (K182R and E269V); however, these mutations do not appear to interfere with 20-carbon chain elongation. The pRAT-4-A7 cDNA had a single, silent mutation at base 726 in comparison to the consensus sequence.

30 The translated amino acid sequence of the consensus TELO1 sequence had 34.0% identity in 265 amino acids with HSELO1

(Figure 83), 34.1% identity in 267 amino acids with MELO4 (Figure 84), 43.4% identity in 244 amino acids with GLELO (Figure 85), and 34.3% identity in 239 amino acids with CEELO (Figure 86).

5       The yeast cells containing the fungal elongase cDNAs also had elevated levels of the monounsaturated fatty acid 18:1n-7, compared to the control strain (Figure 81). Therefore, these results indicated that the identified fungal elongases are capable of utilizing PUFAs as well as monounsaturated fatty acids as substrates. Thus, these fungal sequences TELO1, and their encoded proteins (TELO1p), possess elongase activity independent of substrate specificity.

10      To further confirm the substrate specificity of the fungal elongation enzyme, described above and referred to herein as TELO1, the recombinant yeast strain 334(pRAT-4-A4), 334(pRAT-4-A6), 334(pRAT-4-A7), and 334(pRAT-4-D1) were grown in minimal media containing n-6 fatty acids GLA, AA, or n-3 fatty acids STA, or EPA, or no substrate. The lipid profiles of these yeast cultures, when examined by GC and GC-MS, indicated that there were accumulations of DGLA, ADA, ETA,  $\omega$ 3-docosatetrienoic acid (DTA, C22:4n-3), and DPA, respectively (Figure 87). The levels of these fatty acids were 3.88 - 5.42% (DGLA), 0.18 - 0.75% (ADA), 2.27 - 4.01% (ETA), 0.16 - 1.10% (DTA), and 0.54 - 1.74% (DPA), respectively, of the total fatty acids in the strains containing the TELO1 sequence. These represented 64.0 - 77.2%, 1.0 - 5.0%, 79.3 - 89.6%, 3.8 - 32.6%, and 3.4 - 13.3% conversions of the substrate fatty acids, respectively, to the products elongated by two carbon atoms.

15      The yeast cells expressing the recombinant TELO1 sequence, compared to the control cells, also contained significantly

elevated levels of C18:1n-7 and decreased levels of C16:1n-7 (Figure 87). This finding suggested that the recombinant TELO1 protein (TELO1p) might also be involved in the elongation of monounsaturated fatty acids of 16-carbon length.

5 To further confirm the substrate specificity of TELO1p, the recombinant yeast strain 334(pRAT-4-A7) was grown in minimal media containing 25  $\mu$ M of various PUFAs. The lipid profiles of these various substrates revealed that TELO1p is involved in the elongation of n-6 PUFAs LA, GLA, and AA, but not DGLA (Figure  
10 88). The lipid profiles of these yeast cultures indicated that there were accumulations of C20:2n-6, DGLA, and ADA, respectively, but not C22:3n-6. The levels of these fatty acids were 1.07% (C20:2n-6), 5.84% (DGLA), and 0.76% (ADA), respectively, of the total fatty acids in the lysates of  
15 334(pRAT-4-A7). These represented 23.2%, 78.6%, and 3.5% conversions of the substrate fatty acids, respectively, to the products elongated by two-carbon atoms. TELO1p is also involved in the elongation of n-3 PUFAs ALA, STA, and EPA (Figure 88). The lipid profiles of these yeast cultures indicated that there  
20 were accumulations of ETrA, ETA, and DPA, respectively. The levels of these fatty acids were 2.71% ETrA, 4.19% (ETA), and 1.99% (DPA), respectively, of the total fatty acids in the strain containing the TELO1 sequence. These represented 43.3%, 85.3%, and 11.5% conversions of the substrate fatty acids,  
25 respectively, to the products elongated by two-carbon atoms. When STA was added as a substrate, there was also an accumulation of DTA in the culture. The level of this fatty acid was 0.74%, which represented 15.0% conversion of ETA, the two-carbon chain elongated product from substrate STA. All  
30 results confirmed that the expression of TELO1 from T7091 in

yeast resulted in the elongation of various long-chain PUFAs in n-6 and n-3 fatty acid pathways.

Nutritional Compositions

5       The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

10      I. INFANT FORMULATIONS

A.     Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- 20 -Lactose-free formulation to avoid lactose-associated diarrhea.
- Low osmolality (240 mOs/kg water) to reduce risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 25 -1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- 30 -Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0. 11 % calcium phosphate tribasic, 5 potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and disglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl 10 acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, 15 biotin, sodium selenite, vitamin D3 and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of 20 diarrhea in infants and toddlers.

Features:

- First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
- 25 -Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
- Nutritionally complete to meet the nutritional needs of the infant.
- 30 -Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.
- Lactose-free formulation to avoid lactose-associated diarrhea.

- Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding 5 the absorptive capacity of the damaged gut.
- Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help 10 prevent iron deficiency.
- Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve) 86% water, 4.8% corn syrup, 2.5% sugar 15 (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11 % calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono 20 and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, 25 niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

C. Isomil® SF Sucrose-Free Soy Formula With Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

5

Features:

-Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.

-Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).

-Sucrose free for the patient who cannot tolerate sucrose.

-Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.

-1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

-Recommended levels of vitamins and minerals.

-Vegetable oils to provide recommended levels of essential fatty acids.

-Milk-white color, milk-like consistency and pleasant aroma.

20

Ingredients: (Pareve) 75% water, 11.8% hydrolyzed cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch, 0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride,

carageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide,

30

calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine

hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

5 D. Isomil® 20 Soy Formula With Iron Ready To Feed,  
20 Cal/fl oz.:

Usage: When a soy feeding is desired.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6%  
10 sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4%  
coconut oil, 0.15% calcium citrate, 0. 11% calcium phosphate  
tribasic, potassium citrate, potassium phosphate monobasic,  
potassium chloride, mono- and diglycerides, soy,  
lecithin, carrageenan, ascorbic acid, L-methionine, magnesium  
15 chloride, potassium phosphate dibasic, sodium chloride, choline  
chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl  
acetate, zinc sulfate, L-carnitine, niacinamide, calcium  
pantothenate, cupric sulfate, vitamin A palmitate, thiamine  
chloride hydrochloride, riboflavin, pyridoxine hydrochloride,  
20 folic  
acid, manganese sulfate, potassium iodide, phylloquinone,  
biotin, sodium selenite, vitamin D3 and cyanocobalamin.

E. Similac® Infant Formula:

25

Usage: When an infant formula is needed: if the decision is made  
to discontinue breastfeeding before age 1 year, if a supplement  
to breastfeeding is needed or as a routine feeding if  
breastfeeding is not adopted.

30

Features: .

-Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.

5 -Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.

-Carbohydrate as lactose in proportion similar to that of human milk.

-Low renal solute load to minimize stress on developing organs.

-Powder, Concentrated Liquid and Ready To Feed forms.

10

Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, 15 calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

20

F. Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a nutritionally complete 25 formula developed to provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

30 Features:

- Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).
- Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.
- Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.
- More calcium and phosphorus for improved bone mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

25

G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.:

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: -D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, 5 sodium citrate, ascorbic acid, calcium carbonate, mono and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous 10 sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D3, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or 15 added to the infant formulae described above and to other infant formulae known to those in the art.

## II. NUTRITIONAL FORMULATIONS

20

### A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is 25 lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

#### Patient Conditions:

- 30 -For patients on modified diets  
-For elderly patients at nutrition risk ,

- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

5      Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn),  
Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy  
Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium  
Phosphate Tribasic, Sodium Citrate, Magnesium Chloride,  
Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride,  
10     Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc  
Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum,  
Niacinamide,  
Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin  
A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine  
15     Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate,  
Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B.      ENSURE® BARS:

20     Usage: ENSURE BARS are complete, balanced nutrition for  
supplemental use between or with meals. They provide a  
delicious, nutrient-rich alternative to other snacks. ENSURE  
BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie  
flavor is gluten-free. (Honey Graham Crunch flavor contains  
25     gluten.)

Patient Conditions:

- For patients who need extra calories, protein, vitamins and minerals.
- 30     -Especially useful for people who do not take in enough calories and nutrients.

- For people who have the ability to chew and swallow
- Not to be used by anyone with a peanut allergy or any type of allergy to nuts.

Ingredients: Honey Graham Crunch -- High-Fructose Corn Syrup,  
5 Soy Protein Isolate, Brown Sugar, Honey, Maltodextrin (Corn),  
Crisp Rice (Milled Rice,  
Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran,  
Partially Hydrogenated Cottonseed and Soy Oils, Soy  
Polysaccharide, Glycerine, Whey Protein Concentrate,  
10 Polydextrose, Fructose, Calcium Caseinate, Cocoa  
Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower  
Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil.  
Manufactured in a facility that processes nuts.

15 Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium  
Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride),  
Potassium Chloride, Ascorbic Acid, Ferric  
Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc  
Oxide, Calcium Pantothenate, Copper Gluconate, Manganese  
20 Sulfate, Riboflavin, Beta Carotene, Pyridoxine Hydrochloride,  
Thiamine Mononitrate, Folic Acid, Biotin,  
Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium  
Molybdate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

25

Protein: Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

	Soy protein isolate	74%
30	Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, oils, and soy lecithin.

5	Partially hydrogenated cottonseed and soybean oil	76%
	Canola oil	8%
	High-oleic safflower oil	8%
	Corn oil	4%
	Soy lecithin	4%

10

Carbohydrate: Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

15

	High-fructose corn syrup	24%
	Brown sugar	21%
	Maltodextrin	12%
	Honey	11%
20	Crisp rice	9%
	Glycerine	9%
	Soy Polysaccharide	7%
	Oat bran	7%

25 C. ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used 30 as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN

is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

5 Patient Conditions:

-For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

10

Features:

-Low in saturated fat  
-Contains 6 g of total fat and < 5 mg of cholesterol per serving  
-Rich, creamy taste  
15 -Excellent source of protein, calcium, and other essential vitamins and minerals  
-For low-cholesterol diets  
-Lactose-free, easily digested

20 Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate,  
25 Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine

Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

5 Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates 85%  
10 Soy protein isolate 15%

Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

15 High-oleic safflower oil 40%  
Canola oil 30%  
Soy oil 30%

20 The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values  
25 are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

30 ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla

supreme, chocolate royal, wild berry, and banana), plus VARI-  
FLAVORS® Flavor Pacs in pecan,  
cherry, strawberry, lemon, and orange, help to prevent flavor  
fatigue and aid in patient compliance.

5

Vanilla and other nonchocolate flavors:

Sucrose	60%
Maltodextrin	) 40%

10

Chocolate:

Sucrose	70%
Maltodextrin	30%

15

D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE 20 LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

25 -For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.

-For healthy adults who don't eat right and need extra nutrition.

30 Features:

- Low in fat and saturated fat
- Contains 3 g of total fat per serving and < 5 mg cholesterol
- Rich, creamy taste
- Excellent source of calcium and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

10

French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate

15

Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride), Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium

20

Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

25

Protein:

The protein source is calcium caseinate.

Calcium caseinate                    100%

30

Fat:

The fat source is a blend of two oils: high-oleic safflower and canola.

5	High-oleic safflower oil	70%
	Canola oil	30%

The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT 10 represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the, calories from saturated fatty acids, and < 10% of total 15 calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild 20 sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

25

Vanilla and other nonchocolate flavors:

Sucrose	51%
Maltodextrin	49%

30

Chocolate:

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

5

**Vitamins and Minerals:**

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

10 **Caffeine:**

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

**E. ENSURE PLUS®**

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food  
15 for use when extra calories and nutrients, but a normal  
concentration of protein, are needed. It is designed primarily  
as an oral nutritional supplement to be used  
with or between meals or, in appropriate amounts, as a meal  
replacement. ENSURE PLUS is lactose- and gluten-free. Although  
20 it is primarily an oral nutritional supplement, it can be fed by  
tube.

**Patient Conditions:**

-For patients who require extra calories and nutrients, but a  
25 normal concentration of protein, in a limited volume  
-For patients who need to gain or maintain healthy weight

**Features:**

-Rich, creamy taste  
30 -Good source of essential vitamins and minerals

**Ingredients:**

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein

- 5 Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

15

**Protein:**

The protein source is a blend of two high-biologic-value proteins: casein and soy.

- 20 Sodium and calcium caseinates 84%  
Soy protein isolate 16%

**Fat:**

The fat source is corn oil.

25

- Corn oil 100%

**Carbohydrate:**

- ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, buffer pecan, and eggnog), plus

VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

5      Vanilla, strawberry, butter pecan, and coffee flavors:

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

10

Chocolate and eggnog flavors:

Corn Syrup	36%
Maltodextrin	34%
Sucrose	30%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

20

Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

25      F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may 30 be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

**Patient Conditions:**

-For patients with increased calorie and protein needs, such as following surgery or injury.

5 -For patients with limited volume tolerance and early satiety.

**Features:**

-For supplemental or total nutrition

-For oral or tube feeding

10 -1.5 CalmL,

-High nitrogen

-Calorically dense

**Ingredients:**

15

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium

20 Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,

Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride,

25 Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

**G. ENSURE® POWDER:**

30

Usage: ENSURE POWDER (reconstituted with water) is a low-residue

liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

5

Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- 10 -For patients who need a low-residue diet

Features:

- Convenient, easy to mix
  - Low in saturated fat
  - 15 -Contains 9 g of total fat and < 5 mg of cholesterol per serving
  - High in vitamins and minerals
  - For low-cholesterol diets
  - Lactose-free, easily digested
- 20 Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

**Protein:**

The protein source is a blend of two high-biologic-value proteins: casein and soy.

5

Sodium and calcium caseinates	84%
Soy protein isolate	16%

**Fat:**

10       The fat source is corn oil.

Corn oil	100%
----------	------

**Carbohydrate:**

15       ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

20

**Vanilla:**

Corn Syrup	35%
Maltodextrin	35%
Sucrose	30%

**H. ENSURE® PUDDING**Usage: ENSURE PUDDING is a nutrient-dense supplement providing  
30 balanced nutrition in a nonliquid form to be used with or  
between meals. It is appropriate for consistency-modified diets

(e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

Patient Conditions:

- 5 -For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
- For patients with swallowing impairments

Features:

- 10 -Rich and creamy, good taste
  - Good source of essential vitamins and minerals
  - Convenient-needs no refrigeration
  - Gluten-free
- 15 Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:

- Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5,
- 25 Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

- 30 Protein:

The protein source is nonfat milk.

Nonfat milk 100%

5 Fat:

The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil 100%

10 Carbohydrate:

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors:

20 Sucrose 56%  
Lactose 27%  
Modified food starch 17%

Chocolate:

25 Sucrose 58%  
Lactose 26%  
Modified food starch 16%

30 I. ENSURE® WITH FIBER:

Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It  
5 can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

10

Patient Conditions:

- For patients who can benefit from increased dietary fiber and nutrients

15 Features:

- New advanced formula-low in saturated fat, higher in vitamins and minerals
  - Contains 6 g of total fat and < 5 mg of cholesterol per serving
  - Rich, creamy taste
- 20 -Good source of fiber
- Excellent source of essential vitamins and minerals
  - For low-cholesterol diets
  - Lactose- and gluten-free

25 Ingredients:

Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate,  
30 Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride,

Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate,  
Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

## 10 Protein:

The protein source is a blend of two high-biologic-value proteins-casein and soy.

Sodium and calcium caseinates	80%
15 Soy protein isolate	20%

## Fat:

The fat source is a blend of three oils: high-oleic  
20 safflower, canola, and corn.

High-oleic safflower oil	40%
Canola oil	40%
Corn oil	20%

25 The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from  
30 polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the

calories from saturated fatty acids, and  $\leq$  10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

5 ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

10

Vanilla and other nonchocolate flavors:

Maltodextrin	66%
Sucrose	25%
15 Oat Fiber	7%
Soy Fiber	2%

Chocolate:

20 Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%
Soy Fiber	2%

25 Fiber:

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

30

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

5

J. Oxepa™ Nutritional Product

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It

10 has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil),  $\gamma$ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

15 Caloric Distribution:

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl.oz), to minimize the volume required to meet energy needs.

The distribution of Calories in Oxepa is shown in Table IV.

20

Table IV. Caloric Distribution of Oxepa

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)	25	105.5	28.1
25 Protein (g)	14.8	62.5	16.7
Water (g)	186	785	---

Fat:

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).

30 -The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and

3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table V.

-Oxepa provides a balanced amount of polyunsaturated,  
5 monounsaturated, and saturated fatty acids, as shown in Table VI.

-Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal  
10 tract without emulsification by bile acids.

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

Table V. Typical Fatty Acid Profile

	% Total	g/8 fl oz*	g/L*
	Fatty Acids		
	Caproic (6:0)	0.2	0.18
	Caprylic (8:0)	14.69	13.07
10	Capric (10:0)	11.06	9.87
	Palmitic (16:0)	5.59	4.98
	Palmitoleic	1.82	1.62
	Stearic	1.94	1.64
	Oleic	24.44	21.75
15	Linoleic	16.28	14.49
	$\alpha$ -Linolenic	3.47	3.09
	$\gamma$ -Linolenic	4.82	4.29
	Eicosapentaenoic	5.11	4.55
	n-3-Docosapent-	0.55	0.49
20	aenoic		
	Docosahexaenoic	2.27	2.02
	Others	7.55	6.72

25 Fatty acids equal approximately 95% of total fat.

Table VI. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz 40.1 mg/L

## Carbohydrate:

- 10 -The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).  
   -The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.
- 15 -The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO<sub>2</sub>) production. High CO<sub>2</sub> levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced
- 20 hyperglycemia.  
   -Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The

carbohydrate level in Oxepa is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

- 5 -Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).
- The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.
- Oxepa provides enough protein to promote anabolism and the
- 10 maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on CO<sub>2</sub> production, a high protein diet will increase ventilatory drive.
- 15 -The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.
- The amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.

20

\* Oxepa is gluten-free.

Default settings for the analysis programs**GCG Programs**5       FastA Search

Default parameters:

10       range of interest       Begin=1       END=last protein or nucleic  
acidsearch set                   all of SwissProt (protein) or  
GenEMBL(nucleic acid)

15       word size              =(2) for protein    =(6) for nucleic acid

Expected scores lists scores until E( ) value reaches 2.0

20

TFastA search

Default parameters:

25       range of interest       Begin=1           END=last nucleic acid

search set                   all of GenEMBL

30       word size              wordsize=(2)

Expected scores lists scores until E() value reaches 2.0

35       Pileup

Default parameters:

40       gap creation penalty       gap weight = 5

gap extension penalty       gap length weight = 12

plot figure                   one page plot density =2.7

45

Sequencher Program

Default parameters:



```
-E Cost to extend a gap (zero invokes default behavior) [Integer]
      default = 0

5 -X X dropoff value for gapped alignment (in bits) (zero invokes
  default behavior) [Integer]
      default = 0

-I Show GI's in deflines          [T/F]
10      default = F

-q Penalty for a nucleotide mismatch      (blastn only) [Integer]
      default = -3

15 -r Reward for a nucleotide match      (blastn only) [Integer]
      default = 1

-f Threshold for extending hits      default if zero [Integer]
20      default = 0

-g Perform gapped alignment (not available with tblastx) [T/F]
      default = T

25 -q Query Genetic code to use      [Integer]
      default = 1

-D DB Genetic code      (for blast[nx] only)
 [Integer]
      default = 1

30 -J Believe the query defline      [T/F]
      default = F

-M Matrix          [String]
35      default = BLOSUM62

-W Word size      default if zero [Integer]
      default = 0

40 -z Effective length of the database (use zero for the real size)
  [Integer]
      default = 0

-a Number of processors to use      [Integer]
45      default = site configurable (SeqServer.conf)
```

Allowed and default values for gap open/gap extension cost (-G/-E) parameters:

150

•

## BLOSUM62

	-G	9	8	7	12	11	10
	-E	2	2	2	1	1	1

5

## BLOSUM50

	-G	12	11	10	9	15	14	13	12	18	17	16	15
	-E	3	3	3	3	2	2	2	2	1	1	1	1

10

## PAM250

	-G	13	12	11	10	15	14	13	12	19	18	17	16
	-E	3	3	3	3	2	2	2	2	1	1	1	1

15

## BLOSUM90

	-G	8	7	6	11	10	9
	-E	2	2	2	1	1	1

20

## PAM30

	-G	5	4	3	7	6	5	10	9	8
	-E	3	3	3	2	2	2	1	1	1

25

## PAM70

	-G	6	5	4	8	7	6	11	10	9
	-E	3	3	3	2	2	2	1	1	1

30

CLAIMS:

1. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:5 (Figure 54).  
5
2. The isolated nucleotide sequence of claim 1 wherein said sequence comprises SEQ ID NO:5.
- 10 3. The isolated nucleotide sequence of claims 1 or 2 wherein said sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate.
- 15 4. The nucleotide sequence of claim 1 wherein said sequence is derived from a mammal.
5. The nucleotide sequence of claim 4 wherein said sequence is derived from a mouse.
- 20 6. A purified protein encoded by said nucleotide sequence of claims 1 or 2.
7. A purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 6.  
25
8. A method of producing an elongase enzyme comprising the steps of:  
30

- a) isolating a nucleotide sequence comprising SEQ ID NO:5 (Figure 54) or SEQ ID NO:6 (Figure 58);
  - b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;
  - c) introducing said vector into a host cell under time and conditions sufficient for expression of said elongase enzyme.
- 5
9. The method of claim 8 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.
  - 10
  10. The method of claim 9 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.
  - 15
  11. The method of claim 9 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.
  - 20
  12. The method of claim 11 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp., Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp.
  - 25
  13. The method of claim 12 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.
  - 30

14. The method of claim 13 wherein said yeast cell  
is Saccharomyces cerevisiae.

5       15. A vector comprising: a) a nucleotide sequence  
comprising SEQ ID NO:5 (Figure 54) operably linked to b) a  
promoter.

16. A host cell comprising said vector of claim 15.

10

17. The host cell of claim 16, wherein said host  
cell is selected from the group consisting of  
a eukaryotic cell or a prokaryotic cell.

15

18. The host cell of claim 17 wherein said  
prokaryotic cell is selected from the group consisting of  
E. coli, Cyanobacteria, and B. subtilis.

20

19. The host cell of claim 17 wherein said  
eukaryotic cell is selected from the group consisting of a  
mammalian cell, an insect cell, a plant cell and a fungal  
cell.

25

20. The host cell of claim 19 wherein said fungal  
cell is selected from the group consisting of Saccharomyces  
spp., Candida spp., Lipomyces starkey, Yarrowia spp.,  
Kluyveromyces spp., Hansenula spp., Aspergillus spp.,  
Penicillium spp., Neurospora spp., Trichoderma spp. and  
Pichia spp.

30

21. The host cell of claim 20 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

5

22. The host cell of claim 21 wherein said host cell is Saccharomyces cerevisiae.

10

23. A plant cell, plant or plant tissue comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

15

24. The plant cell, plant or plant tissue of claim 23 wherein said polyunsaturated fatty acid is selected from the group consisting of AA, ADA, GLA and STA.

20

25. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 23.

25

26. A transgenic plant comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

30

27. A transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase, operably linked to a promoter, wherein said DNA sequence comprises SEQ ID NO:5 (Figure 54).

28. A fluid produced by said transgenic, non-human mammal of claim 27 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

5

29. A method for producing a polyunsaturated fatty acid comprising the steps of:

- a) isolating a nucleotide sequence comprising SEQ ID NO:5 (Figure 54);
- 10 b) constructing a vector comprising said isolated nucleotide sequence;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by said isolated nucleotide sequence; and
- 15 d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

20

30. The method according to claim 29, wherein said substrate polyunsaturated fatty acid is selected from the group consisting of GLA, STA, AA, ADA and ALA, and said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega 6$ -docosapentaenoic acid and STA, respectively.

25

31. The method according to claim 29 further comprising the step of exposing said expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

30

32. The method according to claim 31 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA and  $\omega$ 6-docosapentaenoic acid, said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid respectively, and said at least one desaturase is  $\Delta$ 5-desaturase with respect to production of AA or EPA, and  $\Delta$ 4-desaturase with respect to 10 production of  $\omega$ 6-docosapentaenoic acid, and  $\Delta$ 19-desaturase with respect to production of docosahexaenoic acid.

33. The method of claim 32 further comprising the step of exposing said another polyunsaturated fatty acid to 15 one or more enzymes selected from the group consisting of at least one elongase and at least one additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

34. The method of claim 33 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

35. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 29, said another polyunsaturated fatty acid produced according to the method

of claim 31, and said final polyunsaturated fatty acid produced according to the method of claim 33.

36. The nutritional composition of claim 35 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA.

37. The nutritional composition of claim 35 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid.

38. The nutritional composition of claim 35 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid.

39. The nutritional composition of claim 35 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

40. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 29, said another polyunsaturated fatty acid produced according to the method of claim 31, and said final polyunsaturated fatty acid

produced according to the method of claim 33 and 2) a pharmaceutically acceptable carrier.

41. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 29, said another polyunsaturated fatty acid produced according to the method of claim 31 and said final polyunsaturated fatty acid produced according to the 10 method of claim 33.

42. The animal feed of claim 41 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA. 15

43. The animal feed of claim 41 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega$ 6-docosapentaenoic acid and docosahexaenoic acid. 20

44. The animal feed of claim 41 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega$ 3-docosapentaenoic acid and docosahexaenoic acid. 25

45. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method 30 of claim 29, said another polyunsaturated fatty acid

produced according to the method of claim 31 and said final polyunsaturated fatty acid produced according to the method of claim 33.

5       46. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 35 in an amount sufficient to effect said prevention or treatment.

10      47. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:6 (Figure 58).

15      48. The isolated nucleotide sequence of claim 47 wherein said sequence comprises SEQ ID NO:6.

49. A purified protein encoded by said nucleotide sequence of claims 47 or 48.

20      50. An isolated nucleotide sequence corresponding to or complementary to at least about 35% of the nucleotide sequence comprising SEQ ID NO:7 (Figure 72).

25      51. The isolated nucleotide sequence of claim 50 wherein said sequence comprises SEQ ID NO:7.

30      52. The isolated nucleotide sequence of claims 50 or 51 wherein said sequence encodes a functionally active elongase which utilizes a polyunsaturated fatty acid as a substrate.

53. The nucleotide sequence of claim 50 wherein said sequence is derived from *Thraustochytrium aureum*.

5 54. A purified protein encoded by said nucleotide sequence of claims 50 or 51.

10 55. A purified polypeptide which elongates polyunsaturated fatty acids and has at least about 30% amino acid similarity to the amino acid sequence of said purified protein of claim 54.

56. A method of producing an elongase enzyme comprising the steps of:

- 15 a) isolating a nucleotide sequence comprising SEQ ID NO:7 (Figure 72);  
b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;  
c) introducing said vector into a host cell under time  
20 and conditions sufficient for expression of said elongase enzyme.

25 57. The method of claim 56 wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

58. The method of claim 57 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.

30 59. The method of claim 57 wherein said eukaryotic

cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

60. The method of claim 59 wherein said fungal cell  
5 is selected from the group consisting of Saccharomyces  
spp., Candida spp., Lipomyces starkey, Yarrowia spp.,  
Kluyveromyces spp., Hansenula spp., Aspergillus spp.,  
Penicillium spp., Neurospora spp., Trichoderma spp. and  
Pichia spp..

10

61. The method of claim 59 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp.

15

62. The method of claim 61 wherein said yeast cell is Saccharomyces cerevisiae.

20

63. A vector comprising: a) a nucleotide sequence comprising SEQ ID NO:7 (Figure 72) operably linked to b) a promoter.

64. A host cell comprising said vector of claim 63.

25

65. The host cell of claim 64, wherein said host cell is selected from the group consisting of a eukaryotic cell or a prokaryotic cell.

30

66. The host cell of claim 65 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

67. The host cell of claim 65 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

10 68. The host cell of claim 67 wherein said fungal cell is selected from the group consisting of Saccharomyces spp., Candida spp., Lipomyces starkey, Yarrowia spp., Kluyveromyces spp., Hansenula spp., Aspergillus spp.,

Penicillium spp., Neurospora spp., Trichoderma spp. and Pichia spp..

15 69. The host cell of claim 68 wherein said fungal cell is a yeast cell selected from the group consisting of Saccharomyces spp., Candida spp., Hansenula spp. and Pichia spp..

20 70. The host cell of claim 69 wherein said host cell is Saccharomyces cerevisiae.

25 71. A plant cell, plant or plant tissue comprising said vector of claim 63, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell, plant or plant tissue.

30 72. The plant cell, plant or plant tissue of claim 71 wherein said polyunsaturated fatty acid is selected from the group consisting of AA, ADA, GLA and STA.

73. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 72.

5 74. A transgenic plant comprising said vector of claim 63, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

10 75. A transgenic, non-human mammal whose genome comprises a DNA sequence encoding an elongase, operably linked to a promoter, wherein said DNA sequence comprises SEQ ID NO:7 (Figure 72).

15 76. A fluid produced by said transgenic, non-human mammal of claim 75 wherein said fluid comprises a detectable level of at least one elongase or products thereof.

20 77. A method for producing a polyunsaturated fatty acid comprising the steps of:

- a) isolating a nucleotide sequence comprising SEQ ID NO:7 (Figure 72);
- b) constructing a vector comprising said isolated nucleotide sequence;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of an elongase enzyme encoded by said isolated nucleotide sequence; and
- d) exposing said expressed elongase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

78. The method according to claim 77, wherein said substrate polyunsaturated fatty acid is selected from the group consisting of GLA, STA, AA, ADA and ALA, and said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega 6$ -docosapentaenoic acid and STA, respectively.

79. The method according to claim 77 further comprising the step of exposing said expressed elongase enzyme to at least one desaturase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

80. The method according to claim 79 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA and  $\omega 6$ -docosapentaenoic acid, said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega 6$ -docosapentaenoic acid and docosahexaenoic acid respectively, and said at least one desaturase is  $\Delta 5$ -desaturase with respect to production of AA or EPA, and  $\Delta 4$ -desaturase with respect to production of  $\omega 6$ -docosapentaenoic acid, and  $\Delta 19$ -desaturase with respect to production of docosahexaenoic acid.

81. The method of claim 79 further comprising the step of exposing said another polyunsaturated fatty acid to one or more enzymes selected from the group consisting of at least one elongase and at least one additional

desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

82. The method of claim 81 wherein said final  
5 polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega 3$ -docosapentaenoic acid and docosahexaenoic acid.

83. A nutritional composition comprising at least one  
10 polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79, and said final polyunsaturated fatty acid produced according to the method of claim 81.  
15

84. The nutritional composition of claim 83 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega 6$ -docosapentaenoic acid and STA.  
20

85. The nutritional composition of claim 83 wherein said another polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega 6$ -docosapentaenoic acid and docosahexaenoic acid.  
25

86. The nutritional composition of claim 83 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega 3$ -docosapentaenoic acid and docosahexaenoic acid.  
30

87. The nutritional composition of claim 83 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a  
5 dietary substitute.

88. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79, and said final polyunsaturated fatty acid produced according to the method of claim 81 and 2) a pharmaceutically acceptable carrier.  
10  
15

89. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79 and said final polyunsaturated fatty acid produced according to the method of claim 81.  
20

90. The animal feed of claim 89 wherein said product polyunsaturated fatty acid is selected from the group consisting of DGLA, 20:4n-3, ADA,  $\omega$ 6-docosapentaenoic acid and STA.  
25

91. The animal feed of claim 89 wherein said another

polyunsaturated fatty acid is selected from the group consisting of AA, EPA,  $\omega 6$ -docosapentaenoic acid and docosahexaenoic acid.

5        92. The animal feed of claim 89 wherein said final polyunsaturated fatty acid is selected from the group consisting of ADA,  $\omega 3$ -docosapentaenoic acid and docosahexaenoic acid.

10      93. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 77, said another polyunsaturated fatty acid produced according to the method of claim 79 and said final polyunsaturated fatty acid produced according to the method 15 of claim 81.

94. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids  
20 comprising administering to said patient said nutritional composition of claim 83 in an amount sufficient to effect said prevention or treatment.

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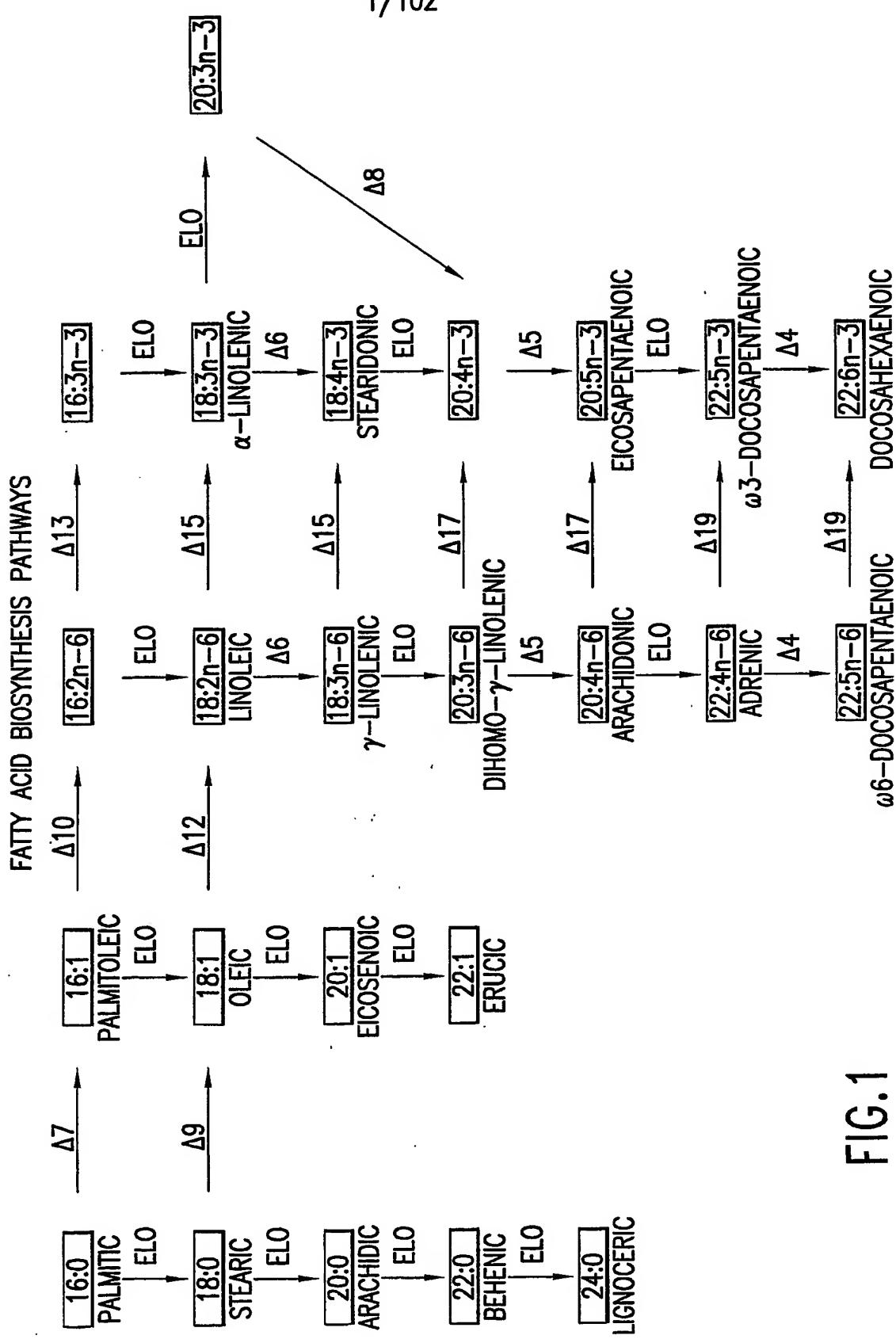


FIG. 1

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Gap Weight: 6 Average Match: 2.912  
Length Weight: 4 Average Mismatch: -2.003

Quality: 50 Length: 84  
Ratio: 0.625 Gaps: 4  
Percent Similarity: 43.038 Percent Identity: 29.114

Match display thresholds for the alignment(s):

| = IDENTITY  
: = 2  
. = 1

jojobakcs x EL02 June 4, 1998 08:23 ..

jojobakcs 24 ATLPNFKSSINLHHVKL.GYHYLISNALFLVFIPLLGASAHLSFSAH 72  
EL02 66 STLPPVLYAITAYYVIIFGGRFLLSKS..KPF.KLNGLFQLHNLVLTSLS 112

jojobakcs 73 LSLLFDLLRRNLLPVVVCNFLFVLLATLHFLTRP 106  
EL02 113 LTLLL.LMVEQLVPIIVQHGLYFAICNIGAWTQP 145

FIG.2

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*S. cerevisiae* EL02 (AA66-145) with *M. alpina* codon bias

S	T	L	P	P	V	L	Y	A	I	T	A	Y	V	I	I	F	G	G	R	F	L	
TCC	ACC	CTC	CCC	CCC	GTC	CTC	TAC	GCC	ATC	ACC	GCC	TAC	GTC	ATC	ATC	TTC	GGT	GGT	CGC	TTC	CTC	
66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88
<-- R0339																						

L	S	K	S	K	P	F	K	L	N	G	L	F	Q	L	H	N	L	V	L	T	S	L
CTC	CTC	AAG	TCC	AAG	CCC	TTC	AAG	CTC	AAC	GGT	CTC	TTC	CAG	CTC	CAC	AAC	CTC	GTC	CTC	ACC	TCC	CTC
89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111

S	L	T	L	L	L	M	V	E	Q	L	V	P	I	I	V	Q	H	G	L	Y	F		
TCC	CTC	ACC	CTC	CTC	CTC	CTC	CTC	ATG	GTC	GAG	CAG	CTC	GTC	CCC	ATC	ATC	GTC	CAG	CAC	GGT	CTC	TAC	TTC
112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	

A	I	C	N	I	G	A	W	T	Q	P
GCC	ATC	TGC	AAC	ATC	GGT	GCC	TGG	ACC	CAG	CCC
135	136	137	138	139	140	141	142	143	144	145

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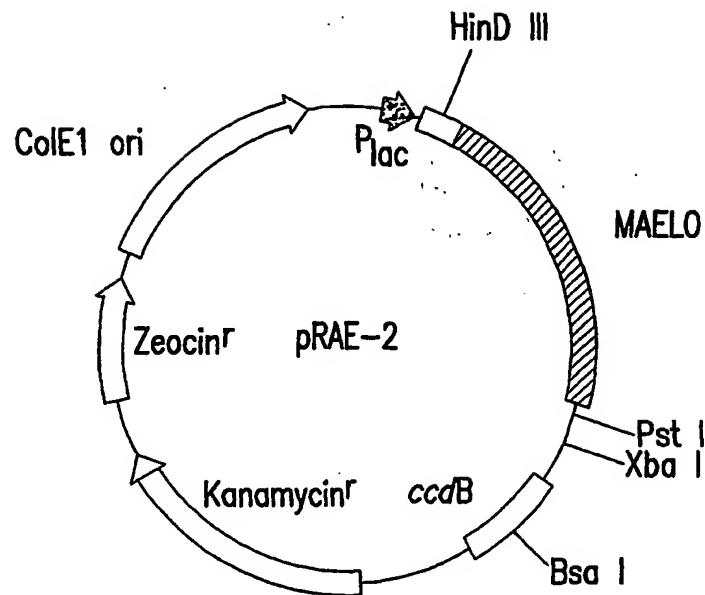


FIG.4A

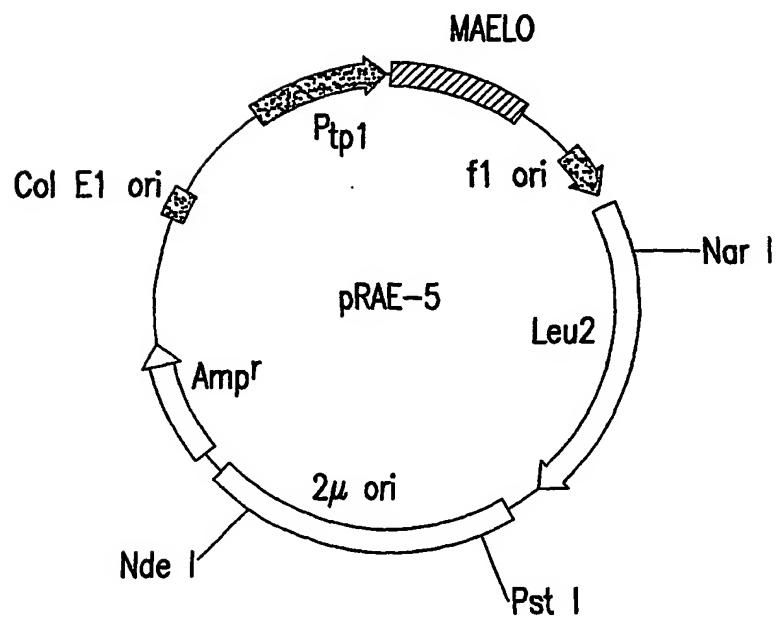


FIG.4B

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pRAE-5      GAATTCAAGG \* \* \* \* \* \* \* \* CATGGCCGCCGCAATCTTGGACAA  
pRAE-6      GAATTCAAGGCATCTCATGGATCCGCCATGGCCGCCGCAATCTTGGACAA  
EcoRI                  BamHI      NcoI

**FIG.5**

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1 ATGGCCGCCG CAATCTTGA CAAGGTCAAC TTGGCATTG ATCAGCCCTT  
51 CGGAATCAAG CTCGACACCT ACTTTGCTCA GGCCTATGAA CTCGTCACCG  
101 GAAAGTCCAT CGACTCCTTC GTCTTCCAGG AGGGCGTCAC GCCTCTCTG  
151 ACCCAGAGAG AGGTGCCAT GTGGACTATC ACTTACTTCG TCGTCATCTT  
201 TGGTGGTCGC CAGATCATGA AGAGCCAGGA CGCCTTCAAG CTCAAGCCCC  
251 TCTTCATCCT CCACAACCTC CTCCGTACGA TCGCGTCCGG ATCGCTGTTG  
301 CTCCCTGTTCA TCGAGAACCT GGTCCCCATC CTCGCCAGAA ACGGACTTTT  
351 CTACGCCATC TGCGACGACG GTGCTGGAC CCAGGCCCTC GAGCTCCTCT  
401 ACTACCTCAA CTACCTGGTC AAGTACTGGG AGTTGGCCGA CACCGTCTTT  
451 TTGGTCCTCA AGAAGAAGCC TCTTGAGTTC CTGCACTACT TCCACCACTC  
501 GATGACCATG GTTCTCTGCT TTGTCAGCT TGGAGGATAC ACTTCAGTGT  
551 CCTGGGTCCC TATTACCCCTC AACTTGACTG TCCACGTCTT CATGTACTAC  
601 TACTACATGC GCTCCGCTGC CGGTGTTCGC ATCTGGTGGA AGCAGTACTT  
651 GACCACTCTC CAGATCGTCC AGTTCTGTTCT TGACCTCGGA TTCATCTACT  
701 TCTGCGCTA CACCTACTTC GCCTTCACCT ACTTCCCCTG GGCTCCCAAC  
751 GTCGGCAAGT GCGCCGGTAC CGAGGGTGCT GCTCTCTTIG GCTGCGGACT  
801 CCTCTCCAGC TATCTCTTGC TCTTTATCAA CTTCTACCGC ATTACCTACA  
851 ATGCCAAGGC CAAGGCAGCC AAGGAGCGTG GAAGCAACTT TACCCCCAAG  
901 ACTGTCAAGT CGGGCGGATC GCCCAAGAAG CCCTCCAAGA GCAAGCACAT  
951 CTAA

## FIG.6

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1 MAAAILDKVN FGIDQPFGIK LDTYFAQAYE LVTGKSIDSF VFQEGVTPLS  
51 TQREVAMWTI TYFVVIFGGR QIMKSQDAFK LKPLFILHNF LLTIASGSLL  
101 LLFIENLVPI LARNGLFYAI CDDGAWTQRL ELLYYLNLYLV KYWELADTVF  
151 LVLKKKPLEF LHYFHHSMTM VLCFVQLGGY TSVSWVPITL NLTvhvfmYY  
201 YYMRSAAAGVR IWWKQYLTTL QIVQFVLDLG FIYFCAYTYF AFTYFPWAPN  
251 VGKCAGTEGA ALFGCGLLSS YLLLFINFYR ITYNAKAKAA KERGSNFTPK  
301 TVKSGGSPKK PSKSKHI\*

FIG. 7

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<p>1      ~~~~~ M N S L V T Q Y A A P L F E R Y P Q L H D Y L P T T E R P F F N I S L W E H F D D V</p> <p>GNS1</p> <p>SUR4</p> <p>MAELO</p>	<p>50</p> <p>V T R V T N G R F Y P S E F Q F I A G E L P L S T I L P P V L Y A I T A Y Y V I I F G G R F I L S K S</p> <p>Y E Y F S . G Y P A E Q . F E F I H N K T E I A N G Y H A V S I I T V Y I I F G G Q A I I L R A I L</p> <p>51</p>	<p>100</p>
<p>101</p> <p>GNS1      K P : F K L I N G [ F Q L H N ] V L T S L S L I I L L M V E Q L V P J I V Q H G L Y F A I G N I G</p> <p>SUR4      N A S P [ K F K L ] F E Y H N F L T S I S L V W L L M L E Q L V P W Y H N G L F W S I C S K E</p> <p>MAELO      D A . . F K L K P L F I L H N F L L T I A S G S S L L F T E N L V P T I L A R N G L F Y A I C D D G</p>	<p>150</p> <p>A W T Q P [ V T Y M N Y I V K F I E E I D T E F L V L K H K K L T F L H T Y H G A T A L [ G Y</p> <p>A F A P X I L V T Y Y L N Y I D T K E Y E L I D T V F L V L R R K K L F L H T Y H G A T A L [ G Y</p> <p>A W T Q R L E L L Y Y L N Y I D T Q R L E L L Y Y L N Y I D T V F L V L K K P L E F L H Y F H H S M T M V L G F</p>	<p>200</p>
<p>151</p> <p>GNS1      T Q L M G I T S I S W V P I S I L N L G V H V V M Y W Y F L A A R G G I R V W W K E W V T R F Q I I Q</p> <p>SUR4      T Q L I G R T S V E W V I L N L G V H V I M Y W Y F L S S C G I R V W W K Q W V T R F Q I I Q</p> <p>MAELO      V Q L G G Y T S V S W V P I T L N L T V H V F M Y Y Y M R S A A G V R I W W K Q Y L T T Q I V Q</p>	<p>250</p> <p>F V L D I G F I Y F A V Y Q K A V H I Y F P I L P H C G D C V G S I I A T E A G C A I I S S Y L V</p> <p>F V L D I D L V F W Y F A T Y T F Y A H K Y D G I L P N K G T C Y G T Q A A A A Y G Y L I I T S Y L L</p> <p>F V L D I G F I Y F C A Y T Y F A F T Y P F W A . P N V G K C A G T E G A A L F G C G L L S S Y L L</p>	<p>300</p>

FIG.8A

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350  
F1 SFY IN Y K R G T K T S R V V K R A H G G V A A K V N E Y N V O L K N V P T P S P S P F1 SFY I Q S Y K K G G K T V K K E S E V S G . S V A S G S S S T G V K I S N T K V S S R K A ~ F1 N Y R I T Y N A K A K A K E R G S N F T P K T V K S G G S P K . K P S K S K H I \* n n n

FIG. 8

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SCORES Init1: 153 Initn: 199 Opt: 495  
 57.4% identity in 549 bp overlap

	150	160	170	180	190	200
MAELO	TCTCGACCCAGAGAGAGGTGCCATGTGGACTATCACTTACCTACTCGTCGTATCTTGGTG					
S78624	CATTAAGCACTTGCCCTGTGCTATAACGCCATCACTGCCTATTACGTTATTATTTTG 5990 6000 6010 6020 6030 6040					
	210	220	230	240	250	260
MAELO	GTCGCCAGATCATGAAGAGGCCAG--GACGCC-TTCAAGCTCAAGCCCTCTTCATCCCTC					
S78624	GTGGCAGGTTTTGTTAAGTAAGTCGAAACCATTAAATTAAATGGCCTTTCCAATTGC 6050 6060 6070 6080 6090 6100					
	270	280	290	300	310	320
MAELO	ACAACCTCCTCCTGACGATCGCGTCC--GGATCGCTTGCTCCTGTTATCGAGAACCT					
S78624	ATAATTGGTTAAC-TTCACCTTCATTGA-CGCTTTATTGCTTATGGTTGAACAATT 6110 6120 6130 6140 6150 6160					
	330	340	350	360	370	380
MAELO	GGTCCCCATCCTGCCAGAAACGGACTTTCTACGCCATCTGCGACGACGGTGCTGGAC					
S78624	AGTGCCTTATTGTTACAGCACGGGTTATACTTCGCTATCTGTAATATTGGTGCTTGGAC 6170 6180 6190 6200 6210 6220					
	390	400	410	420	430	440
MAELO	CCAGCGCCTCGAGCTCCTCTACTACCTCAACTACCTGGTCAAGTACTGGAGTTGGCCGA					
S78624	TCAACCGCTCGTTACATTATATTACATGAATTACATTGTCAAGTTATTGAATTATAGA 6230 6240 6250 6260 6270 6280					
	450	460	470	480	490	500
MAELO	CACCGTCTTTGGTCTCAAGAAGAAGCCTTGTGAGTCTCTGCACTACTTCCACCACTC					
S78624	CACCTTTCTGGTGCTAAAACATAAAAAATTGACATTGGCA-TACTT--ATCA--C 6290 6300 6310 6320 6330 6340					
	510	520	530	540	550	
MAELO	GATGACCATGGTCTGCTTGT---CCAGCTGGAGGATA-CACTTCAGTGTCTGG					
S78624	CATGGCGCTACTGCCATTATGTTACACCAATTGATGGGCACCACATCTATTCTGG 6350 6360 6370 6380 6390 6400					
	560	570	580	590	600	610
MAELO	GTCCCTATTACCCCTCAACTTGACTGTCCACGTCTTATGTACTACTACATGCGCTCC					
S78624	GTCCCTATTCAATTGAAACCTTGGTGTTACGTGGTTATGTATTGGTACTATT--CTTG 6410 6420 6430 6440 6450					
	620	630	640	650	660	670
MAELO	GCTGCC---GGTGTTCGCATCTGGTGGAAAGCAGTACTTGACCACTCTCCAGATCGCCAG					
S78624	GCTGCCAGAGGCATCAGGGCTGGTGGAAAGGAATGGGTACCAAGATTCAAATTATCAA 6460 6470 6480 6490 6500 6510					

FIG.9A

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MAELO	680	690	700	710	720	730
	TTCGTTCTTGACCTCGGATTCACTACTTCTGCCTACACCTACTTCGCCTCACCTAC					
S78624						
	TTTGTTGGATATCGGTTTCATATATTGCTGTCTACCAAAAAGCAGTTCACTTGTAT					
	6520	6530	6540	6550	6560	6570

**FIG.9B**

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HOST (PLASMID)	334(pCCN7875)	334(pYES2)	334(pYX242)	334(pRAE-5)	334(pRAE-6)	334(pYX242)	334(pRAE-5)
ADDED SUBSTRATE	25 μM OA	25 μM OA	25 μM GLA	25 μM GLA	25 μM GLA	NO SUBSTRATE	NO SUBSTRATE
FATTY ACID	LIPID (μg)	LIPID (μg)	LIPID (μg)				
C16:0	11.948	23.601	35.123	92.011	85.160	16.294	25.34
C16:1	30.665	71.217	32.789	315.464	115.456	56.183	113.913
C18:0	6.185	9.704	10.515	22.628	18.879	5.535	11.092
C18:1n-9	35.340	57.429	33.989	154.386	106.881	28.388	51.538
C18:3n-6			48.856	58.084	12.434		
C20:0			0.474	0.710	0.244		
C20:1n-9	(0.375%)* 0.352	(0.309%)* 0.527		1.405	0.867		0.516
C20:3n-6	ND	ND	(0.092%)* 0.226	(0.324%)* 2.504	(0.269%)* 1.006	ND	ND
C22:0				0.460			
C22:1n-9				0.321	0.315		
C24:0					1.825		0.999
TOTAL LIPID	93.760	170.490	245.090	771.690	374.420	112.99	256.52
ND = NOT DETECTED							
*% TOTAL FATTY ACID							

FIG. 10A

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HOST/PLASMID	334(pγγ242)	334(pγγ242)	334(pRAE-5)	334(pRAE-5)	334(pRAE-6)
ADDED SUBSTRATE	25 μM GLA				
FATTY ACID	LIPID (μg)				
C16:0	60.683	61.487	100.998	96.193	66.761
C16:1	79.838	79.586	359.754	220.440	87.359
C18:0	9.784	10.106	15.317	15.165	16.744
C18:1n-9	38.536	39.936	108.472	89.637	71.631
C18:3n-6	17.974	17.833	82.866	56.596	17.766
C20:0			0.510	0.570	
C20:1n-9					
C20:3n-6	(0.136%)* 0.389	(0.130%)* 0.374	(0.336%)* 3.035	(0.401%)* 2.689	(0.353%)* 1.185
C22:0			0.414		
C22:1n-9				0.383	
C24:0			1.513	1.626	
TOTAL LIPID	285.560	288.045	902.560	671.113	335.496
% TOTAL FATTY ACID					

\*% TOTAL FATTY ACID

FIG. 10B

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HOST(PLASMID)	334(pRAE-5/pCGR4)	334(pYX242/pYES2)	HOST(PLASMID)	334(pRAE-5/pCGR4)	334(pYX242/pYES2)
ADDED SUBSTRATE	25 μM GLA	25 μM GLA	ADDED SUBSTRATE	25 μM GLA	25 μM GLA
FATTY ACID	LIPID (μg)	LIPID (μg)	LIPID (μg)	LIPID (μg)	LIPID (μg)
C16:0	41.050	37.169	C16:0	96.986	32.221
C16:1	99.393	100.552	C16:1n-7	209.667	62.757
C18:0	34.432	27.852	C18:0	80.418	14.027
C18:1	110.631	92.786	C18:1n-9	207.104	28.701
C18:3n-6	15.004	7.924	C18:3n-6	25.264	10.543
C20:0	0.643	0.574	C20:0	2.038	
C20:1	1.996	1.684	C20:1n-9	3.591	
C20:3n-6	0.542	0.607	C20:3n-6	1.284	0.326
C20:4n-6	0.579		C20:4n-6	1.394	
C22:0	1.242	2.604	C22:0	1.124	
C24:0	4.754	4.563	C24:0	3.952	
TOTAL LIPID	334	300	TOTAL LIPID	756	197

FIG. 11

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HOST(PLASMID)	334(pYX242)	334(pRAE-5)	334(pRELO-1)	334(pRELO-2)
ADDED SUBSTRATE	25 $\mu$ M GLA			
	25°C/48HRS	25°C/48HRS	25°C/48HRS	25°C/48HRS
FATTY ACID	LIPID ( $\mu$ g)			
C16:0	28.7	76.707	84.424	77.445
C16:1	0.729	2.513	1.532	1.056
C18:0	7.432	15.761	27.17	21.32
C18:1n-9	28.9	77.323	109.419	82.844
C18:3n-6	9.729	29.236	19.085	18.804
C20:0		0.643	0.522	0.537
C20:1n-9		0.77	0.426	0.299
C20:3n-6	(0.185%)* 0.374	(0.279%)* 1.472	(0.153%)* 0.748	(0.200%)* 0.832
C22:0		0.451		
C22:1n-9			0.224	
C24:0			0.918	
TOTAL LIPID	202	527	490	416
% TOTAL FATTY ACID				

FIG. 12

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SCORES      Init1: 156 Initn: 215 Opt: 296  
 Smith-Waterman score: 296; 28.8% identity in 264 aa overlap

	10	20	30	40	50	60	
U61954	RTFKMMDQILGTNFTYEGAKEVARGLEGFSAKLAVGYIATIFGLKYYMKDRKAFDLSTPL						
							:: :::    :   ::   :
MAELO	AQAYELVTGKSIDSFVFQEGVTPLSTQREVAMTITYFVVIFGGRQIMKSQDAFKLK-PL						
	30	40	50	60	70	80	
	70	80	90	100	110	119	120
U61954	NIWNGILSTFSLLGFLFTF-PTLLSVIRKDGFHSHTYSHVSELVTDSTSGYWI-----F						
							::   :::   :   :   :   :   :   :   :   :   :   :   :   :
MAELO	FILHNFLLTIASGSLLLLFIENLVPILARNGL-----FYAICDDGAWTQRLELLYY						
	90	100	110		120	130	
	130	140	150	160	170		
U61954	LWVISKIPELLDTVFIVLRKRPLIFMHWYHHALTGYALVCYHE--DAVHMVW-VWMNY						
							:       :   :   :   :   :   :   :   :   :   :   :   :   :
MAELO	LNLYLVKYWELADTVFLVLKKKPLEFLHYFHHSMT--MVLCFVQLGGYTSWSWPITLNL						
	140	150	160	170	180	190	
	180	190	200	210	220	230	
U61954	IIHAFMYGYLLKSLKVPIPPSVAQAITTSQMVQFA-----VAIFAQVHVSYKHYVEGVE						
							: :   :   :   :   :   :   :   :   :   :   :   :   :   :   :
MAELO	TVHVFMYYYYMRSAAGVRI--WWKQYLTTLQIVQFVL_DLGIYFCAYTYFAFTYFPWAPN						
	200	210	220	230	240	250	
	240	250	260	270	280		
U61954	-GLAYSFRGTAI-GFFMLTTYFYLWIQFYKEHYLKNGGKKYNLAKDQAKTQTKKAN						
							: :   :   :   :   :   :   :   :   :   :   :   :   :
MAELO	VGKCAGTEGAALFGCGLLSSYLLLFINFYRITY---NAKAKAAKERSNFTPCKTVKSGG						
	260	270	280	290	300		
MAELO	SPKKPSKSKHIX						
	310						

FIG.13

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SCORES      Init1: 178 Initn: 178 Opt: 318  
 Smith-Waterman score: 318; 33.0% identity in 188 aa overlap

	50	60	70	80	90	100	
Z68749	SLLTNQDEVFPHIRARRFIQEHFGLFVQMAIAVILVFSIKRFMRDREPQLTALRLWN						
MAELO	ELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFFGRQIMKSQDAFKLKPLFILHN						
	30	40	50	60	70	80	
	110	120	130	140	150	160	
Z68749	FFLSVFSIYGSWTFMFPI--MVQQIRLYGLYCGCCEALSNLPSQAELYWLFLTILSKAVEFV						
MAELO	FLLTIAS--GSLLLFIENLVPILARNGLFYAICDD-GAWTQRLELLYYLNVLKYWELA						
	90	100	110	120	130	140	
	170	180	190	200	210	220	
Z68749	DTFFLVLRKPKLIFLHWYHHMATEVFFCSNYPTPSSQSRGVIVNLVHAFMYPYYFTRS						
MAELO	DTVFLVLKKKPLEFLHYFHHSMTMVLCFVQLGGYTSWSWPITLNLTvhvfmYYYYMRSA						
	150	160	170	180	190	200	
	230	240	250	260	270		
Z68749	MNIKVPAKISMAVTLQLTQF--MCFIYGCTLYYSLATNQARYPSNTPATLQCLSYTL						
MAELO	AGVRIWK--QYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVGKCAGTEGAALFG						
	210	220	230	240	250	260	
	280						
Z68749	HLL						
MAELO	CGLLSSYLLFINFYRITYNAKAKAAKERGSNFTPCKVSGGSPKKPSKSKHIX						
	270	280	290	300	310		

FIG.14

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SCORES      Init1: 30 Initn: 30 Opt: 40  
Smith-Waterman score: 49; 22.1% identity in 86 aa overlap

AF003134	10            20.            30            40 MLYSITRRCYTFFVTSLHFYQLYVTECLEENVIFNVLVNGQSINSRWKD  : : :     :  : : :: : :  :    :::: : 
MAELO	MAAAILDKVNFGIDQPFGIKLDTYFAQA--YELVTGKSIDSFVFQEGVT--PLSTQREV 10            20            30            40            50

FIG. 15

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## Mouse

SCORES Initl: 161 Initn: 191 Opt: 325  
 Smith-Waterman score: 325; 28.8% identity in 285 aa overlap

U97107	10	20	30	39	40	
	MDTSMNFSRGLKMD--LMQPYDFETFQDLRPFLEEVWVSSF-----LIVV					
MAELO	10	20	30	40	50	60
	MAAAILDKVNFIDQPFGIKLDTYFAQAYELVTGKSIDSFVFQEGVTPLSTQREVAMWTI					
U97107	50	60	70	80	90	100
	VYLLLIVVGQTYMRTRKSFSLQRPLILWSFFLAIFSILGTLRMWKFMATVMFTVGLQTV					
MAELO	70	80	90	100	110	
	TYFVVIFGGGRQIMKSQDAFKLKPLFILHNFLLTIAS--GSL-LILFIENLV-PILARNGL					
U97107	110	120	130	140	150	
	CFAIYTDDAVVRFWNSFLFLSKVV--ELGDTAFIILRKRPLIFVHWYHHST--VLLFTS					
MAELO	120	130	140	150	160	170
	FYAICDDGAWTQRLELLYYLNLYLVKYWEADTVFLVLKKKPLEFLHYFHHSMTMVLCFVQ					
U97107	160	170	180	190	200	210
	FGYKNKVPSSGGWF-MTMNFGVHSVMYTYTMKAAKLKHPNLLPMVITSQILQMVLG---					
MAELO	180	190	200	210	220	230
	LGGYTSV---SWVPITLNLTVHVFMYYYYYRSAAGVR--IWWKQYLTTLQIVQFVLDLGF					
U97107	220	230	240	250	260	
	-----TIFGILNYIWRQEKG-CHTTTEHFFWSFMLYGTYFILFAHFFRAYLRPKGVVA					
MAELO	240	250	260	270	280	290
	IYFCAYTYFAFTYFPWAPNVGKAGTEGAALFGCGLLSSYLLLFINFYRITY-NAKAHAA					
U97107	270					
	SKSQX					
MAELO	300	310				
	KERGSNFTPKTVKSGGSPKKPSKSKHIX					

## Human

SCORES Initl: 147 Initn: 147 Opt: 211  
 Smith-Waterman score: 211; 28.7% identity in 150 aa overlap

MAELO	110	120	130	140	150	160
	NLVPILARNGLFYAICDDGAWTQRLELLYYLNLYLVKYWEADTVFLVLKKKPLEFLHYFH					
AC004050	10	20	30			
	SLLVVKDLYLLPLCLPGDTIFIILRKQKLIFLHWYH					
MAELO	170	180	190	200	210	220
	HSMTMVLCFVQLGGYTSVSWVPITLNLTVHVFMYYYYYRSAAGVRIWWK--QYLTTLQIV					
AC004050	40	50	60	70	80	90
	HITVLLYSWYSYKDMVAGGGWFMTMNYGVHAVMSYYALRAAGFRVSRSKFAMFITLSQT					
MAELO	230	240	250	260	270	280
	QFVLDLGFIFYFCAYTYFAFTYFPWAPNVGKAGTEGAALFGCGLLSSYLLLFINFYRITY					
AC004050	100	110	120	130	140	
	QMLMG-----CVVNYLVFC---WMQH-DQCHSHFQNIFWSSLMYLSYLVLFCHFFFAY					

FIG.16

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SCORES Initl: 87 Initn: 218 Opt: 232  
 Smith-Waterman score: 272; 29.7% identity in 232 aa overlap

	40	50	60	70	80	90	
MAELO	SFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFILHNFLLTIASGS						
I05465							:   :     ::   :     :
	20	30	40	50	60	70	
	100	110	120	130	140	150	
MAELO	LLLLFIENLVPILARNGLFYAICDDGAWTQRLELLYYL--NYLVKYWELADTVFLVLKKK						
I05465							:     :: :   :   : ::         :         :         :
	80	90	100	110	120		
	160	170	180	190	200	210	
MAELO	--PLEFLHYFHH-SMTMVLCFVQLGGYTSVSWPITLNLTvhvfmYYYY-MRSAAGVR--						
I05465							:     :     :     :     :         :         :         :
	130	140	150	160	170	180	
	220	230	240	250	260	270	
MAELO	IWWKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVGKCAGTEGAALFGCGLSS						
I05465							:     :     :     :     :         :         :
	190	200	210		220	230	
	280	290	300	310			
MAELO	YLLLFINFYRITYNAKAKAAKERGSNFTPCKTSGGSPKKPSKSKHIX						
I05465							:             :   :   :
	240	250	260	270	280	290	

FIG.17

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1 MGTDQGKTFT WEELAAHNTK DDLLLAIRGR VYDVTKFLSR HPGGVDTLLL  
51 GAGRDVDTPVF EMYHAFGAAD AIMKKYYVGT LVSNELPIFP EPTVFHKTICK  
101 TRVEGYFTDR NIDPKNRPEI WGRYALIFGS LIASYYAQLF VPFWVERTWL  
151 QVVFAIIMGF ACAQVGLNPL HDASHFSVTH NPTWKLGA THDFFNGASY  
201 LVWMYQHMLG HHPYTNIAGA DPDVSTSEPD VRRIKPQKW FVNHNQHMF  
251 VPFLYGLLAF KVRIQDINIL YFVKTNDAIR VNPISTWHTV MFWGGKAFFV  
301 WYRLIVPLQY LPLGVLLLLF TVADMVSSYW LALTFQANHV VEEVQWPLPD  
351 ENGIQKDWA AMQVETTQDY AHDSHLWTI TGSLNYQAVH HLFPNVSQHH  
401 YPDILAIIKN TCSEYKVPLY VKOTFWQAF A SHLEHLRVLG LRPKEE\*

FIG.18

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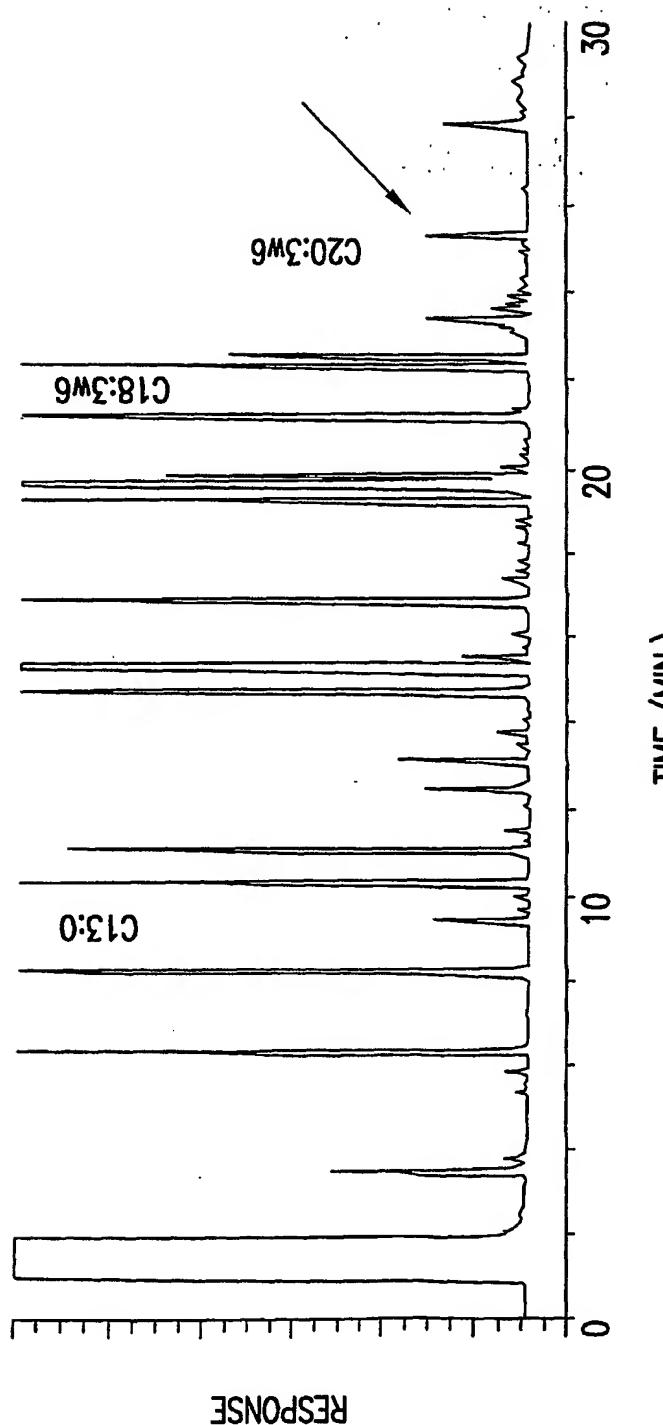


FIG. 19

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FATTY ACID	% TOTAL LIPID			334(MAD708-30)	334(prAE5)
	334(MAD708-10)	334(MAD708-18)	25 μM GLA		
HOST(PLASMID)	334(MAD708-2)	334(MAD708-10)	334(MAD708-18)	334(MAD708-19)	334(MAD708-30)
ADDED SUBSTRATE	25 μM GLA				
TOTAL LIPID ( $\mu$ g)	238.47	307.86	188.51	167.31	207.47
(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)					466.65

FIG.20

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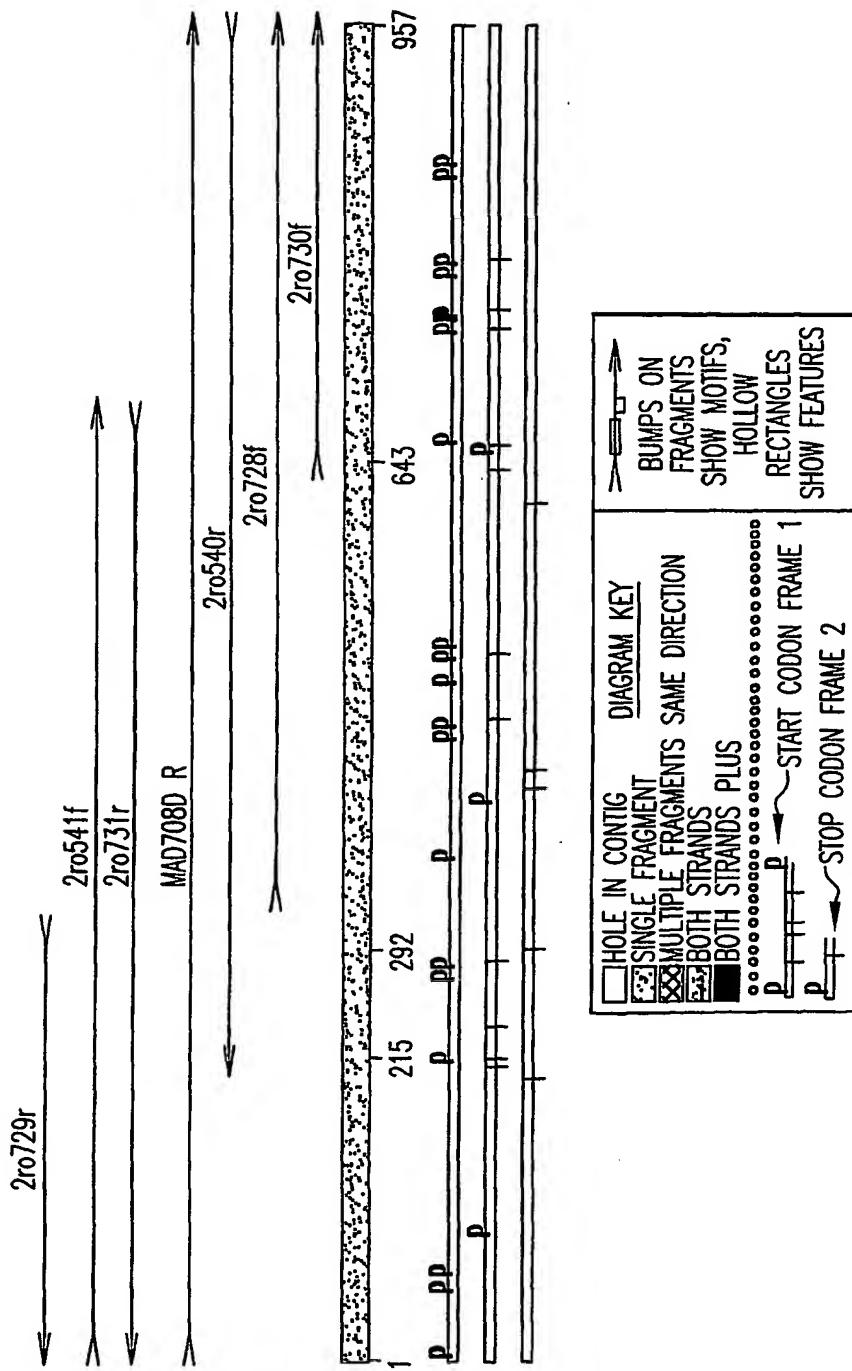


FIG.21

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1 MEAVVNLYQE VMKHADPRIQ GYPLMGSPLL MTSILLTYVY FVLSLGPRIM  
51 ANRKPFQLRG FMIVYNFSLV ALSLYIVYEF LMSGWLSTYT WRCDPVDYSN  
101 SPEALRMVRV AWLFLFSKFI ELMDTVIFIL RKKDGQVTFL HVFHHSVLPW  
151 SWWWGVKIAP GGMGSFHAMI NSSVHVIMYL YYGLSAFGPV AQPYLWKHH  
201 MTAIQLIQFV LVSLHISQYY FMSSCNYQYP VIIHLIWMYG TIFFMLFSNF  
251 WYHSYTKGKR LPRALQQNGA PGIAKVKA\*

**FIG.22**

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1 MESIAPFLPS KMPQDLFMDL ATAIGVRAAP YVDPLEAALV AQAEKYIPTI  
51 VHHTRGFLVA VESPLARELP LMNPFHVLLI VLAYLTVVFV GMQIMKNFER  
101 FEVKTFSLLE NFCLVSISAY MCGGILYEAY QANYGLFENA ADHTFKGLPM  
151 AKMIWLFYFS KIMEFVDTMI MVLKKNNRQI SFLHVYHHSS IFTIWWLVTF  
201 VAPNGEAYFS AALNSFIHVI MYGYYFLSAL GFKQVSFIKF YITRSQMTQF  
251 CMMSVQSSWD MYAMKVLGRP GYPFFITALL WFYMWTMLGL FYNFYRKNAK  
301 LAKQAKADAA KEKARKLQ\*

FIG.23

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HOST(PLASMID)	334(pRPB2)	334(pYES2)
ADDED SUBSTRATE	25 $\mu$ M GLA	25 $\mu$ M GLA
	(n=4)	
FATTY ACID	% TOTAL LIPID	
C16:0	15.65	15.23
C16:1	35.2	38.59
C18:0	5.68	5.55
C18:1n-9	25.55	25.27
C18:3n-6	3.1	6.75
C20:0	0.36	0.14
C20:3n-6	(62.0%) 5.06	(2.6%) 0.18
TOTAL LIPID ( $\mu$ g)	314	247

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.24

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HOST(PLASMID)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)	334(pRPB2)
ADDED SUBSTRATE	25 mM SA	25 mM OA	25 mM LA	25 mM DGLA	25 mM AA
C18:0	C18:1n-9	C182n-6	C20:3n-6	C20:4n-6	C22:4n-6
FATTY ACID					% TOTAL LIPID
C16:0	15.07	14.52	15.74	15.69	16.06
C16:1	33.7	32.37	32.23	25.65	33.65
C18:0	*9.78	5.83	5.61	8.33	4.52
C18:1n-9	31.2	*37.25	26.05	20.15	24.54
C182n-6			*10.4		
C183n-6					
C20:2n-6			0.29		
C20:3n-6				*16.5	
C20:4n-6				0.27	*11.7
C22:4n-6					*7.46
TOTAL LIPID (m)	132	130	171	55	225
					163

FIG.25A

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HOST(PLASMID)	334(prPB2)	334(prPB2)	334(prPB2)
ADDED SUBSTRATE	25 μM ALA	25 μM STA	25 μM EPA
C18:3n-3	C18:4n-3	C20:5n-3	
FATTY ACID	% TOTAL LIPID		
C16:0	17.32	16.01	20.67
C16:1	27.68	34.31	50.7
C18:0	6.75	5.39	6.14
C18:1n-9	28.4	28.54	
C18:5n-3	*8.39		
C18:4n-3		*1.95	
C20:4n-3		(73.2%) 5.33	
C20:5n-3			*10.33
C22:5n-3			0.25
TOTAL LIPID (μg)	114	199	201

\*INDICATES SUBSTRATE ADDED  
 (% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.25B

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HOST(PLASMID)	334(pRPB2+PRPE31)	334(pYES2+pYX242)
ADDED SUBSTRATE	25 μM GLA	25 μM GLA
FATTY ACID	% TOTAL LIPID	
C16:0	15.54	18.26
C16:1	30.16	33.51
C18:0	8.76	5.58
C18:1n-9	27	27.37
C18:3n-6	*2.6	*5.6
C20:0	0.4	0.32
C20:3n-6	(57.4%) 3.55	(2.9%) 0.17
C20:4n-6	(27.6%) 1.32	ND
TOTAL LIPID (μg)	254	258

\*INDICATES SUBSTRATE ADDED  
 (% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

## FIG.26A

HOST(PLASMID)	334(pRPB2+PRPE31)	334(pYES2+pYX242)
ADDED SUBSTRATE	25 μM STA	25 μM STA
FATTY ACID	% TOTAL LIPID	
C16:0	18	16.4
C16:1	28.37	34.78
C18:0	7.42	5.71
C18:1n-9	26.44	30.15
C18:4n-3	*2.93	*4.57
C20:0	0.25	0.17
C20:4n-3	4.13	0.32
C20:5n-3	(39%) 1.87	(2.1%) .10
TOTAL LIPID (μg)	257	304

\*INDICATES SUBSTRATE ADDED  
 (% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

## FIG.26B

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SCORES Initl: 114 Initn: 278 Opt: 278  
 Smith-Waterman score: 308; 30.9% identity in 259 aa overlap

GLELO	40	50	60	70	80	90	99
	VAQAEKYIPTIVHHTRGFLVAVESPLARELPLMNPFHVLLIVLAYLTVFVGMQIMKNFE						
MAELO	20	30	40	50	60	70	
	GIKLDTYFAQAYELVTGKSIDSFVFQEGVTPPLSTQREVAMWTITYFVVIFGGRQIMKSQD						
GLELO	100	110	120	130	140	150	
	RFEVKTFSSLHNFCLVSIAYMCGGILYE--AYQANYGLFENAADHTFKGLPMAKMIWLF						
MAELO	80	90	100	110	120	130	
	AFKLKPLFILHNFLLTIASGSLLLLFIENLVPILARNGLFYAIACDDGAWTQRLELLYYLN						
GLELO	160	170	180	190	200	210	
	YFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAALNSFI						
MAELO	140	150	160	170	180	190	
	YLVKYWELADTVFLVLKK--KPLEFLHYFHHS-MTMVLCFVQLGGYTSVSWVPITLNLT						
GLELO	220	230	240	250		260	
	HVIMYGYYFLSALGFQVSFIKFYITRSQMTQF-----CMMSVQS----SWDMYAM						
MAELO	200	210	220	230	240	250	
	HVFMYYYYMRSAAGVRI--WWKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVG						
GLELO	270	280	290	300	310		
	KVLRGPYPFFITALLWFYMWMLGLFYNFYRKNAKLAKQAKADAAKEKARKLQ						
MAELO	260	270	280	290	300		
	KCAGTEGAALFGCGLLSSYLL---LFINFYR---ITYNAAKAAKERSNFTPCKTVKS						
MAELO	310						
	GGSPKKPSKSKHIX						

FIG.27

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GLELO	1	- M E S T I A P F L P S K M P Q D L F M D L A T A I G V R A A P Y V D P L E A A L V A Q	42
MAELO	1	- - - - -	20
GNS1	1	- - - - - M N S L V T Q Y A A P E F E R Y P Q L H D Y L P T L E R P F F N I S L W	36
SUR4	1	M N T T T S T V I A A V A D Q F Q S E N S S C F L K V H V P S I E N P - F G I E L	42
GLELO	43	A E K Y I P T T I V H H T R G F L V A V E S P L A R E E L P U M N P F H V L L I V L A Y L	85
MAELO	21	L D T Y F A Q A Y E L V T G K S I D S E V F Q E G V T P L S T Q R E V A M W T I T Y F	63
GNS1	37	E H F D D V V T R V T N G R E V P S E F Q F I A G E L P L S T L P P V L Y A I T A Y Y	79
SUR4	43	W P I F S K V F E Y F S G - Y P A E Q F E F I H N K I F I A N G Y H A V S I I I V Y Y	84
GLELO	86	V T V F V G M Q I M K N F E R F E V K T F S E L H N F C L V S I S A Y M C G G I L Y E	128
MAELO	64	V V I F G G R Q I M K S Q D A - F K L K P E F I L H N F L E T I A S G S L L L F I	104
GNS1	80	V V I F G G R F L L S - K S K P F K L N G L F Q L H N L V L T S L S L T L L L M V	120
SUR4	85	I I I F G G Q A I I L R A L N A S P L K F K L L F E I H N L F L T S I S L V L W L L M L	127
GLELO	129	- - A Y Q - - A N Y G L F E N A A D H T F K G L P M A K M I W L F Y F S K I M E F V D	167
MAELO	105	E N L V P I L A R N G L F Y A I C D D G A W T Q R I E L L Y Y L N Y L V K Y W E L A D	147
GNS1	121	E Q L V P I I V Q H G L Y F A I C N I G A W T Q P L V T L Y Y M N Y I V K F I E F I D	163
SUR4	128	E Q L V P M V Y H N G L F W S I C S K E A F A P K L V T L Y Y L N Y L T K F V E L I D	170
GLELO	168	I M I M V L K K N N R Q I S F L H V Y H H S S I F T I W W L V T F V A P N G E A Y F S	210
MAELO	148	T V F L V L K K - K P L E F L H Y F H S M I M V L C F - V Q L G G Y T S V S W V P	187
GNS1	164	T F F L V L K H - K K L T F L H T Y H H G A T A L L C Y - T Q L M G T T S I S W V P	203
SUR4	171	T V F L V L R R - K K L F L H T Y H H G A T A L L C Y - T Q L I G R T S V E W V V	210

FIG.28A

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GLELO	211	A A <u>L</u> <u>N</u> <u>S</u> <u>F</u> <u>I</u> <u>H</u> <u>V</u> <u>I</u> <u>M</u> <u>Y</u> <u>G</u> <u>Y</u> <u>Y</u> <u>F</u> <u>L</u> <u>S</u> <u>A</u> <u>L</u> <u>G</u> <u>F</u> <u>K</u> <u>Q</u> <u>V</u> <u>S</u> <u>F</u> <u>I</u> <u>R</u> <u>F</u> <u>Y</u> <u>I</u> <u>T</u> <u>R</u> <u>S</u> <u>Q</u> <u>M</u> <u>T</u> <u>Q</u> <u>F</u> <u>C</u> <u>M</u>	253
MAELO	188	<u>I</u> <u>T</u> <u>L</u> <u>N</u> <u>L</u> <u>T</u> <u>V</u> <u>H</u> <u>V</u> <u>F</u> <u>M</u> <u>Y</u> <u>Y</u> <u>M</u> <u>R</u> <u>S</u> <u>A</u> <u>G</u> <u>V</u> <u>R</u> <u>-</u> <u>-</u> <u>I</u> <u>W</u> <u>W</u> <u>K</u> <u>Q</u> <u>Y</u> <u>L</u> <u>T</u> <u>T</u> <u>L</u> <u>Q</u> <u>I</u> <u>V</u> <u>Q</u> <u>F</u> <u>V</u> <u>L</u> <u>D</u>	228
GNS1	204	<u>I</u> <u>S</u> <u>L</u> <u>N</u> <u>L</u> <u>G</u> <u>V</u> <u>H</u> <u>V</u> <u>V</u> <u>M</u> <u>Y</u> <u>W</u> <u>Y</u> <u>Y</u> <u>F</u> <u>L</u> <u>A</u> <u>A</u> <u>R</u> <u>G</u> <u>I</u> <u>R</u> <u>-</u> <u>-</u> <u>V</u> <u>W</u> <u>W</u> <u>K</u> <u>E</u> <u>W</u> <u>V</u> <u>T</u> <u>R</u> <u>F</u> <u>Q</u> <u>I</u> <u>I</u> <u>Q</u> <u>F</u> <u>V</u> <u>L</u> <u>D</u>	244
SUR4	211	<u>I</u> <u>L</u> <u>N</u> <u>L</u> <u>G</u> <u>V</u> <u>H</u> <u>V</u> <u>I</u> <u>M</u> <u>Y</u> <u>W</u> <u>Y</u> <u>F</u> <u>L</u> <u>S</u> <u>S</u> <u>C</u> <u>G</u> <u>I</u> <u>R</u> <u>-</u> <u>-</u> <u>V</u> <u>W</u> <u>W</u> <u>K</u> <u>Q</u> <u>W</u> <u>V</u> <u>T</u> <u>R</u> <u>F</u> <u>Q</u> <u>I</u> <u>I</u> <u>Q</u> <u>F</u> <u>L</u> <u>I</u> <u>D</u>	251
GLELO	254	<u>S</u> <u>V</u> <u>Q</u> <u>S</u> <u>S</u> <u>W</u> <u>D</u> <u>M</u> <u>Y</u> <u>A</u> <u>M</u> <u>K</u> <u>V</u> <u>L</u> <u>G</u> <u>R</u> <u>P</u> <u>G</u> <u>Y</u> <u>P</u> <u>F</u> <u>F</u> <u>I</u> <u>T</u> <u>A</u> <u>L</u> <u>L</u> <u>W</u> <u>F</u> <u>Y</u> <u>M</u> <u>W</u> <u>T</u> <u>M</u> <u>L</u> <u>G</u> <u>L</u> <u>F</u> <u>Y</u> <u>N</u> <u>F</u> <u>Y</u> <u>R</u>	296
MAELO	229	<u>L</u> <u>G</u> <u>F</u> <u>I</u> <u>Y</u> <u>F</u> <u>C</u> <u>A</u> <u>Y</u> <u>T</u> <u>Y</u> <u>F</u> <u>A</u> <u>F</u> <u>T</u> <u>T</u> <u>Y</u> <u>F</u> <u>P</u> <u>W</u> <u>-</u> <u>A</u> <u>P</u> <u>N</u> <u>V</u> <u>G</u> <u>K</u> <u>C</u> <u>A</u> <u>G</u> <u>T</u> <u>E</u> <u>G</u> <u>A</u> <u>A</u> <u>L</u> <u>F</u> <u>G</u> <u>C</u> <u>G</u> <u>L</u> <u>L</u> <u>S</u> <u>S</u>	270
GNS1	245	<u>I</u> <u>G</u> <u>F</u> <u>I</u> <u>Y</u> <u>F</u> <u>A</u> <u>V</u> <u>Y</u> <u>Q</u> <u>K</u> <u>A</u> <u>V</u> <u>H</u> <u>L</u> <u>Y</u> <u>F</u> <u>P</u> <u>-</u> <u>I</u> <u>L</u> <u>P</u> <u>H</u> <u>C</u> <u>G</u> <u>D</u> <u>C</u> <u>V</u> <u>G</u> <u>S</u> <u>T</u> <u>T</u> <u>A</u> <u>T</u> <u>F</u> <u>A</u> <u>G</u> <u>C</u> <u>A</u> <u>I</u> <u>I</u> <u>S</u>	286
SUR4	252	<u>L</u> <u>V</u> <u>F</u> <u>V</u> <u>Y</u> <u>F</u> <u>A</u> <u>T</u> <u>T</u> <u>Y</u> <u>T</u> <u>F</u> <u>Y</u> <u>A</u> <u>H</u> <u>K</u> <u>Y</u> <u>L</u> <u>D</u> <u>G</u> <u>I</u> <u>L</u> <u>P</u> <u>N</u> <u>K</u> <u>G</u> <u>T</u> <u>C</u> <u>Y</u> <u>G</u> <u>T</u> <u>Q</u> <u>A</u> <u>A</u> <u>A</u> <u>Y</u> <u>G</u> <u>Y</u> <u>L</u> <u>I</u> <u>T</u> <u>S</u>	294
GLELO	297	K N A K L A K Q A K A D A A <u>W</u> E K A R K L Q	
MAELO	271	<u>Y</u> <u>L</u> <u>L</u> <u>F</u> <u>I</u> <u>N</u> <u>F</u> <u>Y</u> <u>R</u> <u>I</u> <u>T</u> <u>Y</u> <u>N</u> <u>A</u> <u>K</u> <u>A</u> <u>K</u> <u>E</u> <u>R</u> <u>G</u> <u>S</u> <u>N</u> <u>F</u> <u>T</u> <u>P</u> <u>K</u> <u>T</u> <u>V</u> <u>K</u> <u>S</u> <u>G</u> <u>G</u> <u>S</u> <u>P</u> <u>K</u> <u>K</u> <u>P</u> <u>S</u> <u>K</u>	313
GNS1	287	<u>Y</u> <u>L</u> <u>V</u> <u>L</u> <u>F</u> <u>I</u> <u>S</u> <u>F</u> <u>Y</u> <u>I</u> <u>N</u> <u>V</u> <u>Y</u> <u>K</u> <u>R</u> <u>K</u> <u>G</u> <u>T</u> <u>T</u> <u>S</u> <u>R</u> <u>V</u> <u>V</u> <u>K</u> <u>R</u> <u>A</u> <u>H</u> <u>G</u> <u>G</u> <u>V</u> <u>A</u> <u>K</u> <u>V</u> <u>N</u> <u>E</u> <u>Y</u> <u>V</u> <u>N</u> <u>V</u> <u>D</u> <u>L</u>	329
SUR4	295	<u>Y</u> <u>L</u> <u>L</u> <u>F</u> <u>I</u> <u>S</u> <u>F</u> <u>Y</u> <u>I</u> <u>Q</u> <u>S</u> <u>Y</u> <u>K</u> <u>K</u> <u>G</u> <u>K</u> <u>K</u> <u>T</u> <u>V</u> <u>K</u> <u>K</u> <u>E</u> <u>S</u> <u>E</u> <u>V</u> <u>S</u> <u>G</u> <u>S</u> <u>V</u> <u>A</u> <u>S</u> <u>G</u> <u>S</u> <u>S</u> <u>T</u> <u>G</u> <u>V</u> <u>K</u> <u>T</u> <u>S</u> <u>N</u>	337
MAELO	314	S <u>K</u> <u>H</u> <u>I</u>	317
GNS1	330	K <u>N</u> <u>V</u> <u>P</u> <u>T</u> <u>P</u> <u>S</u> <u>S</u> <u>P</u> <u>K</u> <u>P</u> <u>Q</u> <u>H</u> <u>R</u> <u>R</u> <u>K</u> <u>R</u>	347
SUR4	338	T <u>K</u> <u>V</u> <u>S</u> <u>S</u> <u>R</u> <u>K</u> <u>A</u>	345

FIG.28B

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SCORES Init1: 83 Initn: 186 Opt: 271  
Smith-Waterman score: 297; 28.5% identity in 242 aa overlap

	30	40	50	60	70	80
MAELO	YELVTGKSIDSFVQEGVPLSTQREVAMWTITYFVVIFGGGRQIMKSQDAFKLKLPLFILH					
HS1	STYFKALLGPRDTRVKGWFLLDNYIPTFICSVIYLLIVWLGPKYMRNKOPFSCRGILVWY					
	10	20	30	40	50	60
MAELO	90	100	110	120	130	140
	NFLLTIASGSLLLLFIENLVPILARNGLFYAI CDDLGAWTQRLELLYYL--NYLVKYWELA					
HS1	:  :      :    :: :  :   : : ::     :     :					
	NLGLTLLS--LYMFCELVTGVWEGKYNFFCQGTRTAGESDMKIIRVLWWYYSKLIIEFM					
	70	80	90	100	110	120
MAELO	150	160	170	180	190	200
	DTVFLVLKK--KPLEFLHYFHH-SMTMVLCFVQLGGYTSVSWPITLNLTVHVFMYYYY-					
HS1	:: :  : :    :      :   : :  :      :  :					
	DTFFFILRKNNHQITVLHVYHASMLNIIWWFVMNWVPCGHSYFGATLNSFIHVLMYSYYG					
	130	140	150	160	170	180
MAELO	210	220	230	240	250	260
	MRSAAAGVR--IWNKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVGKAGTEGA					
HS1	:  : ::  :   :     ::      : :  :        :					
	LSSVPSMRPYLWWKKYITQGQLLQFVLTI-IQTSCGVI-----W-P----CTFPLGW					
	190	200	210	220		230
MAELO	270	280	290	300	310	
	ALFGCGLLSSYLLLFINFYRITYNAKAKAAKERGSNFTPCKTVSGGSPKKPSKSCHKI					
HS1	:   :             : : ::					
	LYFQIGYIMISLIALFTNFYIQTYNKKGASRRKDHLKDHQNGSMAAVNGHTNSFSPLENNV					
	240	250	260	270	280	290
HS1	KPRKLRKDX					
	300					

FIG.29

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SCORES Init1: 88 Initn: 208 Opt: 272  
 Smith-Waterman score: 279; 28.2% identity in 266 aa overlap

	30	40	50	60	70	80
MAELO	QAYELVTGKSIDSFVFQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFI					
HS2	VNLYQEVMKHADPRIQGYPLMGSPLLMTSILLTYVYFVLSLGPR-IMANRKPFQLRGFMI					
	10	20	30	40	50	60
	90	100	110	120	130	140
MAELO	LHNFLLTIASGSLLLLFIEN--LVPILAR-NGLFYAICDDGAWTQRLELLYYLNVLKYW					
HS2	VYNFSLVALSLYIVYEFMSGWLSTYTWRCDPVDSNSPEALRMVRVAWLFLFS---KFI					
	70	80	90	100	110	120
	150	160	170	180	190	
MAELO	ELADTVFLVLKKK--PLEFLHYFHHSMT---MVLCFVQLGGYTSVSWPITLNLTVHF					
HS2	ELMDTVIFILRKKDGQVTFLHVFHHSVLPWSWWGVKIAPGGMGSFHAM---INSSVHVI					
	130	140	150	160	170	
	200	210	220	230	240	
MAELO	MYYYYYMRSAAGV---RIWWKQYLTTLQIVQFVL---DLGFIYF---CAYTYFAFTYFPW					
HS2	MYLYYGLSAFGPVAQPYLWWKHMATAIQLIQFVLVSLHISQYYFMSSCNQYPVIIHLIW					
	180	190	200	210	220	230
	250	260	270	280	290	300
MAELO	APNVGKAGTEGAALFGCGLLSYLLLFINFYRITYNAKAKAAKERSNFTPCKTVSGGS					
HS2	-----MYG-----TIFFMLFSNFWYHSYTKGKRLPRALQQNGAPGIAKVKAN					
	240	250	260	270		
	310					
MAELO	PKKPSKSKHI					
HS2	X					
	280					

FIG.30

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SCORES Init1: 88 Initn: 207 Opt: 223  
 Smith-Waterman score: 236; 30.4% identity in 191 aa overlap

	100	110	120	130	140	150	
MAELO	LLLLFIENLVPILARNGLFYAICDDGAWTQRLELLYYLNLYLVKYWELADTVFLVLKKKP-						
	20	30	40	50	60	70	
MM2	IVYEFLMSGWLSTYTWRCDPIDFSNSPEALRMVRVAWLFMLSKVIELMDTVIFILRKKD						
	160	170	180	190	200	209	
MAELO	-LEFLHYFHHSMTMVLCF----VQLGGYTSVSVPITLNLTIVHFMYYYYMRSAAAGV---						
	80	90	100	110	120	130	
MM2	QVTFLHVFFHHSVLPWSWWNGIKIAPGGMGSFHAM---INSSVHVVMYLYYGLSALGPVAQ						
	210	220	230	240	250	260	
MAELO	-RIWWKQYLTLQIVQFVL---DLGFIYF---CAYTYFAFTYFPWAPNVGKCAGTEGAAL						
	140	150	160	170			
MM2	PYLWWKKHMTAIQLIQFVLVSLHISQYYFMPSCNYQYPVIIHLW-----M						
	270	280	290	300	310		
MAELO	FGCGLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSCHKI						
	180	190	200	210			
MM2	YG----TIFFILFSNFWYHSYTKGKRLPRAVQQNGAPATTKVKA						

## FIG.31

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SCORES Init1: 51 Initn: 115 Opt: 168  
 Smith-Waterman score: 168; 30.4% identity in 115 aa overlap

MAELO	30	40	50	60	70	80
	YELVTGKSIDSFVFQEGVTPPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPFLIH					
AI225632	::  :   :  ::  :: : ::  :					
	NAFLDNMFGPRDSRVRGWFLLDSYLPTFILTITYLLSIWLGNKYMKNRPALSLRGILTLY					
	20	30	40	50	60	70
MAELO	90	100	110	120	130	140
	NFLLTIASGSLLLLFIENLVPILARNGLFYAICDD---GAWTQRLELLYYLNVLVKYWE					
AI225632	: : :  : : :    : :  :  ::       :  :					
	NLAITLLSAYMLVELI-----LSSWEGGYNLQCQNLDSDAGEGDVRVAKVLWYYYFSKLVE					
	80	90	100	110	120	
MAELO	150	160	170	180	190	200
	LADTVFLVLKKK--PLEFLHYFHHSMTMVLCFVQLGGYTSWSWPITLNLTVHVFYYYY					
AI225632	:   : :  :   :     :  :					
	FLDTIFFVLRKKANQITFLHVYHHASMFNI					
	130	140	150			

**FIG.32**

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SCORES Frame: (3) Init1: 332 Initn: 332 Opt: 384  
 40.3% identity in 144 aa overlap

	80	90	100	110	120	130
GLELO	LIVLAYLVTVFVGMQIMKKNFERFEVKTFSLHNFCVLVSISAYMCGGILYEAYQANYGL-F					
	: : :: :     : :: :::::: :					
AI815960	LYNLGITLLSAYMLAELILSTWEGGYNLQC					
			10	20	30	
	140	150	160	170	180	190
GLELO	ENAADHTFKGLPMMAKMIWLFLYFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWL					
	:: :: : :  :  :     :  :  :  :  :  :  :  :  :  :  :  :  :					
AI815960	QDLTSAGEADIRVAKVLWWYYFSKSVEFLDTIFFVLRKCTSQTFLHVYHASMFNIWWC					
	40	50	60	70	80	90
	200	210	220	230	240	250
GLELO	VTFVAPNGEAYFSAALNSFIHVIMYGYYFLSAL-GFKQVSFIKFYITRSQMTQFCMMSVQ					
	:: : :      :  :       : ::  :    : : :					
AI815960	VLNWIPCGQSFFGPTLNSFIHILMYSYYGLSVFPSMHKLWKKYLTAQQLVQF					
	100	110	120	130	140	
	260	270	280	290	300	310
GLELO	SSWDMYAMKVLGRPGYPFFITALLWFYMWTLGLFYNFYRKNAKLAKQAKADAKEKARK					

FIG.33

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SCORES Init1: 316 Initn: 384 Opt: 477  
 Smith-Waterman score: 477; 34.2% identity in 240 aa overlap

GLELO	50	60	70	80	90	100
	AQAEKYIPTIVHHTRGFLVAVESPLARELPLMNPFHVLIVLAYLTVFVGMQIMKNFER					
HS1	10	20	30	40	50	
	MEHFDASLSTYFKALLGPRDTRVKGWFLLDNYIPTFICSVIYLLIVWLGPKYMRNKQP					
GLELO	110	120	130	140	150	159
	FEVKTFSLHNFCCLSISAYMCGGILYEAYQANYGLF-ENAADHTFKGLPMAKMIWLFYF					
HS1	60	70	80	90	100	110
	FSCRGILVVYNLGLTLLSLYMFCELVTGVWEGKYNFFCQGTRTAGESDMKIIRVLWWYYF					
GLELO	160	170	180	190	200	210
	SKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAAALNSFIHV					
HS1	120	130	140	150	160	170
	SKLIEFMDTFFFILRKNNHQITVLHVYHHASMLNIWWFVMNWPCGHSYFGATLNSFIHV					
GLELO	220	230	240	250	260	270
	IMYGYYFLSAL-GFKQVSFIKFYITRSQMTQFCMMSVQSSWDMYAMKVVLGRPYPFFITA					
HS1	180	190	200	210	220	230
	LMYSYYGLSSVPSMRPYLWWKKYITQGQLLQFVLTIQTS-----CGVIWPCTFPLGWLY					
GLELO	280	290	300	310		
	LLWFYMWTLGLFYNFYRK--NAKLAKQAKADAACEKARKLQ					
HS1	240	250	260	270	280	290
	FQIGYMLISLIALFTNFYIQTYNIKKGASRRKDHLKDHQNGSMAVNIGHTNSPLENNVKP					

FIG.34

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SCORES Init1: 80 Initn: 114 Opt: 178  
 Smith-Waterman score: 178; 28.8% identity in 146 aa overlap

	140	150	160	170	180	190
GLEL0	FENAADHTFKGLPMMAKMIWLFYFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWW					
AC004050	: :: :  :::         :::   DTIFIILRK--QKLIFLHWYHHITVLLYSW					
	10	20				

	200	210	220	230	240	250
GLEL0	LVTFVAPNGEAYFSAALNSFIHVIMYGYYFLSALGFQVSFIKFYITRSQMTQFCMMSVQ					
AC004050	:  ::  :  :  :        : : :      :  :     YSYKDMVAGGGWF-MTMNYGVHAVMYSYYALRAAGFRVSRKFAMFITLSQITQMLMGCVV					
	30	40	50	60	70	80

	260	270	280	290	300	310
GLEL0	SSWDMYAMKVLGRPGYPFFITALLW--FYMWTLGLFYNFYRKN--AKLAKQAKADAACE					
AC004050	: :  : :   ::  ::: ::     : : : :   :  : NYLVFCWMQ--HDQCHSHF-QNIFWSSLMYLSYLVLFCHFFFSEAYIGKMRKTTKAEX					
	90	100	110	120	130	140

GLEL0      KARKLQ

# FIG.35

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SCORES Init1: 288 Initn: 288 Opt: 399  
Smith-Waterman score: 399; 34.6% identity in 211 aa overlap

GLELO	80	90	100	110	120	130
	LLIVLAYLTVFVGMQIMKNFERFEVKTSLLHNFCLVSIAYMCGGILYEAYQANYGLF					
MM2				::::      :   : : : :: ::		
				IVYNFSLVILSLYIVYEFLMSGWLSTYTWR		
					10	20
						30
GLELO	140	150	160	170	180	190
	ENAAD--HTFKGLPMAKMIWLFYFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIW					
MM2	CDPIDFSNSPEALRMVRVAWLFLMSKVIELMDTVIFILRKDGQVTFLHVFHHSVLPWSW					
	40	50	60	70	80	90
GLELO	200	210	220	230	240	250
	WLVTFVAPNGEAYFSAALNSFIHVIMGYYFLSALGFQVSFI--KFYITRSQMTQFCMM					
MM2	WWGIKIAPGGMGSFHAMINSSVHVVLYYGLSALGPVAQPYLWWKKHMTAIQLIQFVVL					
	100	110	120	130	140	150
GLELO	260	270	280	290	300	309
	SVQSSWDMYAMKVLRPGYPFFITALLWFYMWTLGLFYNF----YRKNAKLAKQAKADA					
MM2	::  ::    :    :   :  ::       :  :  :  ::  :					
	SLHIS-QYYFMPSCNYQ-YPVIIH-LIWMYGTIFFILFSNFWYHSYTKGKRLPRAVQQNG					
	160	170	180	190	200	
GLELO	310					
	AKEKARKLQ					
MM2						
	APATTKVKAN					
	210					

FIG. 36

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SCORES Init1: 160 Initn: 227 Opt: 269  
Smith-Waterman score: 269; 35.3% identity in 119 aa overlap

	50	60	70	80	90	100	GLELO	
PTIVHHTRGFLVAVESPLARELPLMNPFHVLLIVLAYLTVFVGMQIMKNFERFEVKTFS	:::::::: :     :   :   :							
AI225632	NEVNAFLDNMFGRPRDSRVRGWFLLDSYLPFTILTITYLLSIWLGKYMKNRPALSLRGIL	10	20	30	40	50	60	
GLELO	LLHNFCLVSIAYMCGGILYEAYQANYGLFENAADHTFKG-LPMAK-MIWLFYFSKIMEF	110	120	130	140	150	160	
AI225632	TLYNLAITLLSAYMLVELILSSWEGGYNLQCQNLD SAGEGDVRVAKVLVW-YYFSKLVEF	70	80	90	100	110	120	
GLELO	VDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLTVFVAPNGEAYFSAALNSFIHVIMYGYY	170	180	190	200	210	220	
AI225632	LDTIFFVLRKKANQITFLHVYHHASMFNI	130	140	150				

**FIG. 37**

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SCORES Init1: 64 Initn: 129 Opt: 233  
 Smith-Waterman score: 239; 23.7% identity in 279 aa overlap

	20	30	40	50	60	70
GLELO	FMDLATAIGVRAAPYVDPLEAALVAQAEKYIPTIVHHTRGFLVAVESPLAREL-----PL					
U97107	.: :::  ::::  : : :					
	10	20	30			
	80	90	100	110	120	129
GLELO	MNPFHV--LLIVLAYLVTVFVGMQIMKNFERFEVKTFSSLHNFCLVSIAYMCGGILYE					
U97107	::: : :: :					
	40	50	60	70	80	
	130	140	150	160	170	180
GLELO	YQAN---YGLFENAADHTFKGLPMMAKMIW--LFYFSKIMEFVDTMIMVLKKNNRQISFL					
U97107	:: : : :: :					
	90	100	110	120	130	140
	190	200	210	220	230	240
GLELO	HVYHHSSI--FTIWLVTVFVAPNGEAYFSAALNSFIHVIMYGYYFLSALGFQVSFIKFY					
U97107	: :					
	150	160	170	180	190	200
	250	260	270	280	290	
GLELO	ITRSQMTQFCMMSVQSSWDYAMKVLG--RPGYPFFITALLWFYMWMLGLFYR--FYRK					
U97107	: :					
	210	220	230	240	250	260
	300	310				
GLELO	NAKLAKQAKADAACEKARKLQ					
U97107	:: : : : :					
	270					

FIG.38

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SCORES Init1: 100 Initn: 205 Opt: 271

Smith-Waterman score: 271; 30.7% identity in 218 aa overlap

	60	70	80	90	100	110	
GLELO	TRGFLVAVESPLARELPLMNPFHVLLIVLAYLVTVFVGMQIMKKNFERFEVKTFSSLHNFC						
	30	40	50	60	70	80	
U68749 (F56H11.4)	ATHGPKNFPDAEGRKFFADHFDTIQASILYMVVFGTWKWMRNRQPFQLTIPLNIWNFI						
	120	130	140	150	160		
GLELO	LVSISAYMCGGILYEAYQ--ANYGL---FENAADHTFKGLPMAMKIWLFYFSKIMEFVDT						
	90	100	110	120	130	140	
U68749 (F56H11.4)	LAASFIAAGAVKMTPEFFGTIANKGIVASYCKVFDFT-KG-ENGYWWLFMASKLFELVDT						
	170	180	190	200	210	220	
GLELO	MIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAALNSFIHVIMYGYYFLS						
	150	160	170	180	190		
U68749 (F56H11.4)	IFLVLRK--RPLMLFLHWYHHILTMIIYAWYSHPLTP-GFNRYGIYLNFVVHAFMYSYYFLR						
	230	240	250	260	270	280	
GLELO	ALGFKQVSFIKFYITRSQMTQFCMMSVQSSWDMYAMKVLGRP-GYPFFITALLWFYMWTM						
	200	210	220	230	240	250	
U68749 (F56H11.4)	SMKIRVPGFIAQAITSLQIVQFIISCAVL AHLGYLMHFTNANCDFEPSVFKLAVFMDTTY						
	290	300	310				
GLELO	LGLFYNFYRKNAKLAKQAKADAKEKARKLQ						
	260	270	280				
U68749 (F56H11.4)	LALFVNFFLQSYVLRGKGDKYKAVPKKNN						

FIG.39

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SCORES Init1: 189 Initn: 264 Opt: 358  
 Smith-Waterman score: 358; 28.7% identity in 296 aa overlap

MAELO	10	20	30	40	50	59
	MAAAILDKVNFIDQPFKGKLDTYFAQAYELVTGKSIDSFVFQEGVTPLSTQREVAMW-T					
	::: : :   :	: : :: : :		:: : :   : :		
U68749 (F56H11.4)	MAQHPLVQRLLDVKFDT--KRFVAIATHGPKNFPDAEGRKFFADHFDVTIQAS					
	10	20	30	40	50	
	60	70	80	90	100	110
MAELO	ITYFVVIFGGRQIMKSQDAFKLK-PLFILHNFLLTIASGSLLLLFIENLVPILARNGLFY					
	:   :   : :   : : ::   :           :   : : : :   :					
U68749 (F56H11.4)	ILYMWVVFGTKWFMRNRQPFQLTIPLNIW-NFILAAFSIAGAVKMTPEFFGTIANKGIVA					
	60	70	80	90	100	110
MAELO	120	130	140	150	160	170
	AICDDGAWTQRLELLYYLNLY-KYWELADTVFLVLKKKPLEFLHYFHHSMTMVLCFVQL					
	:   :   : : :   :   :   :   :   :   :   :   :   :   :					
U68749 (F56H11.4)	SYCKVFDFTKGENGYWWLFLMASKLFLVELVDTIFLVLRKRPMLFLHWYHHILTMiyAWYSH					
	120	130	140	150	160	170
MAELO	180	190	200	210	220	230
	GGYTSVSWVPITLNLTvhvfmY-YYyMRSAAgVRI--WWKQYLTLQIVQFVLDLGFIYF					
	: :     :   :     :   :   :   :   :   :   :   :   :   :					
U68749 (F56H11.4)	PLTPGFNRYGIYLNfVvHAFMYSYFLRSMK-IRVPGFIAQAITSLQIVQFIISCAVLAH					
	180	190	200	210	220	
MAELO	240	250	260	270	280	
	CAYT-YFAFTYFPWAPNVGKAGTEGAALFGCGLLSSYLLLFINFYRITY----NAKAK					
	:   :   : :   :     :   :   :   :   :   :   :   :					
U68749 (F56H11.4)	LGYLMHFTNANCDFEPSVFKLA-----VF---MDTTYLALFVNFFLQSvLRGGKDyK					
	230	240	250	260	270	280
MAELO	290	300	310			
	AAKERGSNFTPKTVKSGGSPKKPSKSKHI					
	: : :					
U68749 (F56H11.4)	AVPKKKNN					

FIG.40

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SCORES Init1: 77 Initn: 155 Opt: 264  
 Smith-Waterman score: 264; 27.2% identity in 206 aa overlap

	40	50	60	70	80	90
GLELO	AALVAQAEKYIPTIVHHTRGFLVAVESPLARELPLMNPFHVLILIVLAYLVTVFVGMQIMK					
DM1	PTKMINMDISVTPNYSYIFDFENDFIHQTRKWMLENWTWVFFYYCGIYMLVIFGGQHFMQ					
	10	20	30	40	50	60
	100	110	120	130	140	150
GLELO	NFERFEVKTFSSLHNFCLVSIISAYMCGGILYEAYQA--NYGLFENAADHTF--KGKPMAK					
DM1	NRPRFQLRGPLIWNTLLAMFSIMGAARTAPELIHVLRHYGLFHSVCVPSYIEQDRVCGF					
	70	80	90	100	110	120
	160	170	180	190	200	210
GLELO	MIWLFYFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAA					
DM1	WTWLFVLSKLPRLGDTIFIVLRK--QPLIFLHWYHHITVLIYSWF-SYTEYTSSARWFIV					
	130	140	150	160	170	180
	220	230	240	250	260	270
GLELO	LNSFIHVIMYGYYFLSALGFKQVSFIKFYITRSQMTQFCMMSVQSSWDAMYAMKVLGRPGY					
DM1	MNYCVHSVMYSYYALKAARFNPPRFISMIIITSLQLAQMIIGCAINVWANGFLKTHGTXSC					
	190	200	210	220	230	240
	280	290	300	310		
GLELO	PFFITALLWFYMWMLGLFYNFYRKNAKLAKQAKADAKEKARKLQ					
DM1	HISQRNINLSIAMSSYFVLFARFFYKAYLAPGGHKSRRMA					
	250	260	270	280		

FIG.41

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SCORES Init1: 181 Initn: 279 Opt: 328  
 Smith-Waterman score: 328; 30.0% identity in 237 aa overlap

	40	50	60	70	80	90
MAELO	VTGKSIDSFVQEGVTPLSTQREVAMWTITYFVVIFGGRQIMKSQDAFKLKPLFILHNFL  :: :   : :: :::  : : : :					
DM1	IFDFENDFIHQQRTRKWMLENWTWFYYCGIYMLVIFGGQHFMQNRPRLRGPLIIWNTL 30            40            50            60            70            80					
	100	110	120	130	140	149
MAELO	LTIASGSLLLLFIENLVPILARNGLFYACDDGAWTQ-RLELLY-YLNLYLVKYWEADTV  :::              : : :  :    :::  :       : :  :        :					
DM1	LAMFSIMGAARTAPELIHVLRHYGLFHSVCVPSYIEQDRVCGFWTWLFVLSKLPLEGDTI 90            100            110            120            130            140					
	150	160	170	180	190	200
MAELO	FLVLKKKPLEFLHYFHHSMTMVLCFVQLGGYTS-VSWVPITLNLTWHVFYMYYYMRSAAG  :  : :    :  :   ::: : :     :    :         :					
DM1	FIVLRKQPLIFLHWYHHITVLIYSWFSYTEYTSSARWF-IVMNYCVHSVMSYYALKaar 150            160            170            180            190            200					
	210	220	230	240	250	260
MAELO	VRI--WWKQYLTTLQIVQFVLDLGFIYFCAYTYFAFTYFPWAPNVGKCAGTEGAALFGCG : : : :  :  :   :: :  :  : :  :  :  :  :					
DM1	FNPPRFISMIIITSLQLAQMIIG-----CAINVWANGFLK-THGTXSCHISQRNINLSIA 210            220            230            240            250					
	270	280	290	300	310	
MAELO	LLSSYLLLFINFYRITYNAKAKAAKERGSNFTPKTVKSGGSPKKPSKSKHI :    :    : :    : :					
DM1	MYSSYFVLFARFFYKAYLAPGGHKSRM 260            270            280					

FIG.42

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1 ATGGAACATT TTGATGCATC ACTTAGTACC TATTCAAGG CATTGCTAGG  
51 CCCTCGAGAT ACTAGAGTAA AAGGATGGTT TCTTCTGGAC AATTATATAC  
101 CCACATTTAT CTGCTCTGTC ATATATTTAC TAATTGTATG GCTGGGACCA  
151 AAATACATGA GGAATAAACAA GCCATTCTCT TGCCGGGGGA TTTTACTGGT  
201 GTATAACCTT GGACTCACAC TGCTGTCTCT GTATATGTTC TGTGAGTTAG  
251 TAACAGGAGT ATGGGAAGGC AAATACAAC TCTTCTGTCA GGGCACACGC  
301 ACCGCAGGAG AATCAGATAT GAAGATTATC CGTGTCTCT GGTGGTACTA  
351 CTTCTCCAAA CTCATAGAAT TTATGGACAC TTTCTCTTC ATCCTGCAGCA  
401 AGAACAAACCA CCAGATCACG GTCCCTGCACG TCTACCACCA TGCCCTCGATG  
451 CTGAACATCT GGTGGTTGT GATGAACCTGG GTCCCTGCG GCCACTCTTA  
501 TTTTGGTGCC ACACCTAATA GCTTCATCCA CGTCCTCATG TACTCTTACT  
551 ATGGTTTGTGTC GTCAGTCCCT TCCATGCGTC CATACTCTG GTGGAAGAAG  
601 TACATCACTC AGGGGCAGCT GCTTCAGTTT GTGCTGACAA TCATCCAGAC  
651 CAGCTGCGGG GTCATCTGGC CGTGCACATT CCCTCTGGT TGGTTGTATT  
701 TCCAGATTGG ATACATTATT TCCCTGATTG CTCTCTTCAC AAACCTCTAC  
751 ATTCAAGACT ACAACAAGAA AGGGGCCTCC CGAAGGAAAG ACCACCTGAA  
801 GGACCACCCAG AATGGGTCCG TGGCTGCTGT GAATGGACAC ACCAACAGCT  
851 TTTCACCCCT GGAAAACAAT GTGAAGCCAA GGAAGCTGCG GAAGGATTGA  
901 AGTCAAAGAA TTGA

FIG.43

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1 MEHFDASLST YFKALLGPRD TRVKGWFL LD NYIPTFIC SV IYLLIVWLGP  
51 KYMRNKQPFS CRGILVVYNL GLTLLSLYMF CELVTGVWEG KYNFFCQGTR  
101 TAGESDMKII RVLWWYYFSK LIEFMDTFFF ILRKNNHQIT VLHVYHHASM  
151 LNIWWFVMNW VPCGHSYFGA TLNSFIHVLM YSYYGLSSVP SMRPYLNWKK  
201 YITQGQLLQF VLTIIQTSCG VIWPCTFPLG WLYFQIGYII SLIALFTNFY  
251 IQTYNKKGAS RRKDHLKDHQ NGSVAAVNGH TNSFSPLENN VKPRKLRKD\*

FIG.44

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HOST(PLASMID)	334(pYX242)	334(pRAE-58-A1)	334(pYX242)	334(pRAE-58-A1)
ADDED SUBSTRATE	25 $\mu$ M GLA	25 $\mu$ M GLA	25 $\mu$ M AA	25 $\mu$ M AA
FATTY ACID	% TOTAL FATTY ACID	% TOTAL FATTY ACID	% TOTAL FATTY ACID	% TOTAL FATTY ACID
C18:3n-6	4.40	2.71	0.03	0.04
<b>C20:3n-6</b>	0.09	<b>(50.34%)* 2.75</b>	0.02	0.02
C20:4n-6			7.48	3.97
<b>C22:4n-6</b>			ND	<b>(23.35%)* 1.21</b>
C16:1n-7	41.11	34.72	41.49	35.07
C18:1n-7	1.85	11.33	2.01	<b>11.57</b>
C20:1n-7	0.04	<b>1.48</b>	0.04	<b>1.62</b>
C18:1n-9	15.60	15.66	15.16	14.57
C20:1n-9	0.06	<b>0.22</b>	0.06	<b>0.23</b>
C18:1n-5	0.11	<b>0.62</b>	0.12	<b>0.58</b>
<b>TOTAL LIPID</b>	<b>370</b>	<b>969</b>	<b>359</b>	<b>514</b>
<b>*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)</b>				

FIG.45

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1 ATGGCTCAGC ATCCGCTCGT TCAACGGCTT CTCGATGTCA AATTGACAC  
51 GAAACGATT GTGGCTATTG CTACTCATGG GCCAAAGAAT TTCCCTGACG  
101 CAGAAGGTCG CAAGTTCTTT GCTGATCACT TTGATGTTAC TATTCAAGGCT  
151 TCAATCCTGT ACATGGTCGT TGTGTTCGGA ACAAAATGGT TCATGCGTAA  
201 TCGTCAACCA TTCCAATTGA CTATTCACCT CAACATCTGG AATTTCATCC  
251 TCGCCGCATT TTCCATCGCA GGAGCTGTCA AAATGACCCC AGAGTTCTTT  
301 GGAACCATTG CCAACAAAGG AATTGTCGCA TCCTACTGCA AAGTGTGTTGA  
351 TTTCACGAAA GGAGAGAATG GATACTGGGT GTGGCTCTTC ATGGCTTCCA  
401 AACTTTTCGA ACTTGTTGAC ACCATCTTCT TGTTCTCCG TAAACGTCCA  
451 CTCATGTTCC TTCAGTGTGA TCACCATATT CTCACCATGA TCTACGCCG  
501 GTACTCTCAT CCATTGACCC CAGGATTCAA CAGATACGGA ATTATCTTA  
551 ACTTTGTCGT CCACGCCCTTC ATGTACTCTT ACTACTTCCT TCGCTCGATG  
601 AAGATTGCGG TGCCAGGATT CATCGCCCAA GCTATCACAT CTCTCAAAT  
651 CGTTCAATTG ATCATCTT GCGCCGTTCT TGCTCATCTT GGTTATCTCA  
701 TGCACTTCAC CAATGCCAAC TGTGATTTCG AGCCATCAGT ATTCAAGCTC  
751 GCAGTTTCA TGGACACAAC ATACTGGCT CTTTCGTCA ACTTCTTCCT  
801 CCAATCATAT GTTCTCCGCG GAGGAAAAGA CAAGTACAAG GCAGTGCCAA  
851 AGAAGAAGAA CAACTAA

FIG.46

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1 MAQHPLVQRL LDVKFDTKRF VAIATHGPKN FPDAEGRKFF ADHFDVTIQA  
51 SILYMVVFG TKWFMNRQP FQLTIPLNIW NFILAAFSIA GAVKMTPEFF  
101 GTIANKGIVA SYCKVFDFTK GENGYWWLF MASKLFEVD TIFLVLRKRP  
151 LMFLHWYHHI LTMIYAWYSH PLTPGFNRYG IYLNFVVHAF MYSYYFLRSM  
201 KIRVPGFIAQ AITSLQIVQF IISCAVLAHL GYLMHFTNAN CDFEPSVFKL  
251 AVFMDTTYLA LFVNFFLQSY VLRGGKDKYK AVPKKKNN

FIG.47

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HOST(PLASMID)	334(pYX242)	334(pRET-21)	334(pRET-22)
ADDED SUBSTRATES	50 $\mu$ M GLA + 50 $\mu$ M AA	50 $\mu$ M GLA + 50 $\mu$ M AA	50 $\mu$ M GLA + 50 $\mu$ M AA
FATTY ACID	% TOTAL FATTY ACID	% TOTAL FATTY ACID	% TOTAL FATTY ACID
C16:0	9.22	12.46	9.9
C16:1	0.09	0.18	0.13
C18:0	1.46	2.41	1.49
C18:1n-9	4.03	4.92	3.91
C18:3n-6	10.02	11.89	8.69
C20:3n-6	(1.28%)* <b>0.13</b>	(11.1%)* <b>1.48</b>	(19.4%)* <b>2.09</b>
C20:4n-6	46.98	28.87	35.25
C20:4n-6	0	0	0
TOTAL LIPID (mg)	212	174	187
*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)			

FIG.48

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1 ATGAACATGT CAGTGGTAC TTTACAAGAA TATGAATTG AAAAGCAGTT  
51 CAACGAGAAT GAAGCCATCC AATGGATGCA GGAAAATCGG AAGAAATCTT  
101 TCCTGTTTC TGCTCTGTAT GCTGCCTTA TATTGGTGG TCGGCACCTA  
151 ATGAATAAAC GAGCAAAGTT TGAACTGAGG AAGCCATTAG TGCTCTGGTC  
201 TCTGACCCTT GCAGTCTTCA GTATATTGG TGCTCTTCGA ACTGGTGCTT  
251 ATATGGTGTA CATTGGATG ACCAAAGGCC TGAAAGCAGTC AGTTTGAC  
301 CAGGGTTTT ACAATGGACC TGTCAGCAA TTCTGGCTT ATGCATTTGT  
351 GCTAAGCAAA GCACCCGAAC TAGGAGATAC AATATTCAATT ATTCTGAGGA  
401 AGCAGAAGCT GATCTTCCTG CACTGGTATC ACCACATCAC TGTGCTCCTG  
451 TACTCTGGT ACTCCTACAA AGACATGGTT GCCGGGGGAG GTTGGTTCAT  
501 GACTATGAAC TATGGCGTGC ACGCCGTGAT GTACTCTTAC TATGCCTTGC  
551 GGGCGGCAGG TTTCCGAGTC TCCCAGGAAGT TTGCCATGTT CATCACCTTG  
601 TCCCAGATCA CTCAGATGCT GATGGGCTGT GTGGTTAACT ACCTGGTCTT  
651 CTGCTGGATG CAGCATGACC AGTGTCACTC TCACTTTCAAG AACATTTCT  
701 GGTCCCTCACT CATGTACCTC AGCTACCTTG TGCTCTCTG CCATTTCTTC  
751 TTGAGGCCT ACATCGGCAA AATGAGGAAA ACAACGAAAG CTGAATAG

FIG.49

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1 MNMSVLTQE YEFEKQFNEN EAIQWMQENW KKSFLFSALY AAFIFGGRHL  
51 MNKRAKFELR KPLVLWSLTL AVFSIFGALR TGAYMVKILM TKGLKQSVC  
101 QGFYNGPVSK FWAYAFVLSK APELGDTIFI ILRKQKLIFL HWYHHITVLL  
151 YSWYSYKDMV AGGGWFMTMN YGVHADMYSY YALRAAGFRV SRKFAMFITL  
201 SQITQMLMGC VVNYLVFCWM QHDQCHSHFQ NIFWSSLMYL SYLVLFCHFF  
251 FEAYIGKMRK TTKAE\*

**FIG.50**

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HOST	334	334	334	334	334	334	334	334	334	334	334	334
PLASMID	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58
SUBSTRATE	GLA	GLA	AA	AA	STA	STA	EPA	EPA	OA	OA	ALA	ALA
CONCENTRATION	25 μM	25 μM	25 μM	25 μM	25 μM	25 μM	25 μM	25 μM	25 μM	25 μM	25 μM	25 μM
					% TOTAL LIPID							
C18:1n-9	18.75	12.96	16.95	12.76	16.06	14.48	19.55	13.78	29.42	23.06	ND	14.58
C18:1n-7	2.00	18.49	2.30	18.70	1.45	13.26	2.75	13.62	2.50	16.42	1.87	13.76
C18:1n-5	0.29	1.63	0.24	1.61	0.33	0.97	0.32	1.10	0.30	1.64	0.28	1.18
C18:3n-6	4.61	2.02	0.04	0.04	0.02	0.09	0.06	0.05	0.02	0.05	0.01	0.01
C18:3n-3	0.02	0.08	0.02	0.07	0.01	0.03	0.04	0.05	0.02	0.08	14.74	14.08
C18:4n-3	ND	ND	ND	ND	7.01	2.65	ND	ND	ND	ND	ND	ND
C20:1n-9	0.10	0.77	0.11	0.70	0.15	0.55	0.15	0.46	0.27	2.25	0.10	0.57
C20:1n-7	0.08	8.45	0.10	8.06	0.04	3.95	0.14	4.48	0.10	(8.9%)9.35	0.06	3.53
C20:3n-6	0.17	(78.3%)7.29	0.01	0.07	ND	0.04	ND	ND	ND	ND	ND	ND
C20:3n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:4n-6	ND	ND	22.07	8.40	ND	0.07	ND	ND	ND	ND	ND	ND
C20:4n-3	ND	ND	ND	ND	0.25	(79.2%)10.07	ND	ND	ND	ND	ND	ND
C20:5n-3	ND	ND	0.01	ND	0.18	0.08	8.21	2.63	ND	0.02	ND	ND
C22:4n-6	ND	ND	ND	(42.7%)6.26	ND	ND	ND	ND	ND	ND	ND	ND
C22:5n-3	ND	ND	ND	ND	ND	0.18	ND	(71.7%)6.66	ND	ND	ND	ND
TOTAL LIPID	158	104	144	112	324	209	178	94	148	87	243	315

( % CONVERSION ) = PRODUCT / ( SUBSTRATE + PRODUCT )

ND = NOT DETECTED

FIG. 51

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	HOST	334	334	334	334	334	334	334	334	334	334
PLASMID	pYY242	pRAE-58	pYY242	pRAE-58	pYY242	pRAE-58	pYY242	pRAE-58	pYY242	pRAE-58	pYY242
SUBSTRATE	GLA	GLA	GLA	AA	AA	AA	AA	EPA	EPA	EPA	EPA
CONCENTRATION	25 $\mu$ M	25 $\mu$ M	100 $\mu$ M	100 $\mu$ M	25 $\mu$ M	25 $\mu$ M	100 $\mu$ M	100 $\mu$ M	25 $\mu$ M	25 $\mu$ M	100 $\mu$ M
						% TOTAL LIPID					
C18:1n-9	23.82	21.49	18.49	17.41	22.09	19.23	17.45	18.44	24.78	21.28	19.42
C18:1n-7	2.52	18.35	1.71	11.82	2.54	18.77	1.78	12.67	2.64	19.48	1.79
C18:1n-5	0.15	1.13	0.10	0.54	0.15	1.23	0.10	0.63	0.15	1.18	0.09
C18:3n-6	6.10	2.38	23.30	14.46	0.04	0.02	0.04	0.02	0.04	0.02	0.01
C20:1n-9	0.08	0.83	0.05	0.48	0.10	1.18	0.04	0.56	0.10	1.30	0.06
C20:1n-7	0.10	5.75	0.07	3.09	0.11	9.49	0.05	3.62	0.10	9.94	0.08
C20:3n-6	0.15	(62.0%)3.95	0.31	(39.8%)9.56	0.02	ND	0.04	ND	0.02	0.01	0.01
C20:4n-6	ND	ND	0.01	ND	11.76	7.68	28.39	21.02	0.02	ND	0.01
C20:5n-3	ND	ND	ND	ND	0.03	0.02	0.10	0.07	4.79	2.04	26.47
C22:4n-6	ND	ND	ND	ND	ND	(27.5%)2.91	0.01 (15.7%)1.90	ND	ND	ND	ND
C22:5n-3	ND	ND	ND	ND	ND	ND	0.03	0.02 (70.3%)4.82	0.04 (45.7%)11.50		
TOTAL LIPID	230	419	590	576	249	332	1014	961	372	390	1323
											1065

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

ND = NOT DETECTED

FIG. 52

58/102

HOST	334	334	334	334	334	334	334	334	334	334	334	334	334	334	334
PLASMID	pYX242	pRAE-58	pYX242												
SUBSTRATE	PA	PA	SA	SA	ARA	ARA	BA	BA	PTA	PTA	OA	OA	EA	EA	EA
CONCENTRATION	25 $\mu$ M														
% TOTAL LIPID															
C16:0	24.17	17.23	11.22	7.90	7.74	7.98	7.62	7.11	17.28	11.04	16.06	12.76	14.37	11.98	
C16:1n-7	39.83	33.83	30.62	20.56	21.61	19.81	21.34	22.89	50.06	39.43	40.95	30.06	43.34	29.51	
C16:1n-5	0.30	0.74	0.29	0.58	0.17	0.47	0.18	0.59	0.38	0.80	0.34	0.68	0.37	0.71	
C18:0	1.90	1.50	35.82	38.10	1.12	0.89	1.03	0.88	1.90	1.44	1.82	1.43	1.51	1.23	
C18:1n-9	15.36	14.11	11.52	10.88	8.29	10.03	8.09	10.25	14.55	13.86	20.12	21.37	14.12	15.15	
C18:1n-7	1.36	11.44	0.90	8.72	0.69	8.51	0.69	8.58	1.30	12.76	1.30	13.79	1.21	12.66	
C18:1n-5	0.11	0.78	0.08	0.69	0.08	0.54	0.06	0.61	0.19	0.76	0.10	0.90	0.15	0.84	
C20:0	0.15	0.17	0.09	0.12	52.07	41.48	ND	ND	ND	ND	ND	ND	0.17	0.23	
C20:1n-9	0.09	0.45	0.05	0.30	0.03	ND	0.06	0.28	0.05	0.38	0.18	0.58	7.47	10.97	
C20:1n-7	0.20	2.84	ND	1.52	0.05	1.43	0.14	1.60	0.07	2.76	0.12	2.08	ND	2.30	
C22:0	0.43	0.56	0.29	0.22	0.31	0.19	52.91	38.43	ND	ND	ND	ND	0.32		
C24:0	0.59	1.39	0.36	0.85	0.45	0.71	0.53	1.14	0.45	1.63	0.66	1.02	0.56	0.79	
TOTAL LIPID	297	272	573	542	558	846	585	519	464	295	306	448	309	648	

ND = NOT DETECTED

FIG. 53A

59/102

HOST	334	334	334	334	334	334	334	334	334
PLASMID	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58	pYX242
SUBSTRATE	LA	LA	GLA	GLA	DGLA	AA	AA	ADA	ADA
CONCENTRATION	25 $\mu$ M								
% TOTAL LIPID									
C18:1n-9	15.27	16.83	14.85	15.58	13.62	16.24	15.08	15.64	16.18
C18:1n-7	1.21	13.53	1.22	11.80	1.16	12.63	1.18	11.70	1.30
C18:1n-5	0.13	0.95	0.20	0.73	0.12	0.72	0.14	0.59	0.12
C18:2n-6	4.09	4.85	0.09	0.07	0.07	0.04	0.04	0.04	0.03
C18:3n-6	ND	ND	4.66	2.33	ND	ND	ND	ND	ND
C20:1n-9	0.07	2.60	0.07	0.33	0.07	0.33	0.04	0.27	0.08
C20:1n-7	0.10	0.18	0.14	1.65	0.08	1.68	0.12	1.58	0.12
C20:2n-6	ND (13.2%)	0.74	ND						
C20:3n-6	ND	ND	ND (51.4%)	2.46	6.37	7.86	ND	0.03	ND
C20:4n-6	ND	ND	ND	ND	ND	0.09	6.49	5.77	ND
C22:4n-6	ND	ND	ND	ND	ND	ND	(27.1%)	2.14	10.91
C24:0	0.59	1.61	0.64	1.12	0.69	0.79	0.52	0.77	0.54
TOTAL LIPID	333	373	260	392	260	672	553	690	706
									440

(%CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)  
ND = NOT DETECTED

FIG. 53B

60/102

HOST	334	334	334	334	334	334	334	334	334	334
PLASMID	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58	pYX242	pRAE-58
SUBSTRATE	ALA	ALA	STA	STA	EPA	EPA	DPA	DPA	DPA	DPA
CONCENTRATION	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M
					% TOTAL LIPID					
C18:1n-9	17.21	17.36	16.85	17.71	16.45	16.93	17.08	16.68	18.36	18.77
C18:1n-7	1.29	12.20	1.15	11.38	1.23	11.48	1.33	11.61	1.46	13.72
C18:1n-5	0.14	0.68	0.12	0.57	0.12	0.54	0.12	0.63	0.13	0.79
C18:3n-3	4.42	3.61	ND	0.03	ND	0.03	ND	0.03	ND	0.03
C18:4n-3	ND	0.13	3.04	1.38	ND	0.13	ND	0.13	ND	0.17
C20:1n-9	0.09	0.33	0.11	0.34	0.05	0.31	0.09	0.30	0.13	0.34
C20:1n-7	0.13	1.55	0.05	1.38	0.23	1.89	0.18	1.73	0.15	1.76
C20:3n-3	0.06	(22.2%)1.03	ND	ND	ND	0.11	ND	ND	ND	ND
C20:4n-3	ND	ND	0.06 (61.9%)2.24	ND	ND	ND	ND	ND	ND	ND
C20:5n-3	ND	ND	0.05	0.05	7.43	4.88	ND	ND	0.07	ND
C22:4n-3	ND	ND	ND	0.39	ND	ND	ND	ND	ND	ND
C22:5n-6	ND	ND	ND	ND	ND	ND	0.28	0.41	ND	ND
C22:5n-3	ND	ND	ND	ND	(39.5%)3.19	3.99	5.94	ND	ND	ND
C24:0	0.43	0.73	0.33	0.73	0.45	0.84	0.64	1.07	0.68	0.77
C24:5n-3	ND	ND	ND	ND	ND	0.08	ND	0.06	ND	ND
TOTAL LIPID	696	729	911	710	719	703	602	642	397	684

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

ND=NOT DETECTED

FIG. 53C

61/102

1 ATGGAGCAGC TGAAGGCCTT TGATAATGAA GTCAATGCTT TCTTGGACAA  
51 CATGTTTGGAA CCACGAGATT CTCGAGTTCG CGGGTGGTTC CTGCTGGACT  
101 CTTACCTTCC CACCTTCATC CTCACCATCA CGTACCTGCT CTCGATATGG  
151 CTGGGTAACA AGTACATGAA GAACAGGCCT GCTCTGTCTC TCAGGGGCAT  
201 CCTCACCTTG TATAACCTCG CAATCACACT TCTTCTGCG TATATGCTGG  
251 TGGAGCTCAT CCTCTCCAGC TGGGAAGGAG GTTACAACTT GCAGTGTCA  
301 AATCTCGACA GTGCAGGAGA AGGTGATGTC CGGGTAGCCA AGGTCTGTG  
351 GTGGTACTAC TTCTCCAAAC TAGTGGAGTT CCTGGACACG ATTTCTTG  
401 TTCTACGAAA AAAGACCAAT CAGATCACCT TCCTTCATGT CTATCACCAC  
451 GCGTCCATGT TCAACATCTG GTGGTGTGTT TTGAAC TGGA TACCTTGTGG  
501 TCAAAGCTTC TTTGGACCCA CCCTGAACAG CTTTATCCAC ATTCTCATGT  
551 ACTCCTACTA CGGCCTGTCT GTGTTCCCCT CCATGCACAA GTACCTTG  
601 TGGAAAGAAGT ACCTCACACA GGCTCAGCTG GTGCAGTTCG TACTCACCAT  
651 CACGCACACG CTGAGTGCCG TGGTGAAGCC CTGTGGCTTC CCCTTGGCT  
701 GTCTCATCTT CCAGTCTTCC TATATGATGA CGCTGGTCAT CCTGTTCTTA  
751 AACTTCTATA TTCAGACATA CCGGAAAAAG CCAGTGAAGA AAGAGCTGCA  
801 AGAGAAAGAA GTGAAGAATG GTTTCCCCAA AGCCCACCTTA ATTGTGGCTA  
851 ATGGCATGAC GGACAAGAAG GCTCAATAA

FIG.54

62/102

1 MEQLKAFDNE VNAFLDNMFG PRDSRVRGWF LLDSYLPPTFI LTITYLLSIW  
51 LGNKYMKNRP ALSLRGILTL YNLAITLLSA YMLVELILSS WEGGYNLQCQ  
101 NLDLSAGEGDV RVAKVLWWYY FSKLVEFLDT IFFVLRKKTN QITFLHVYHH  
151 ASMFNIWWCV LNWI PCGQSF FGPTLNSFIH ILMYSYYGLS VFPSMHKYLW  
201 WKKYLTQAQL VQFVLTITHT LSAVVKPCGF PFGCLIFQSS YMMLVILFL  
251 NFYIQTYRKK PVKKE LQEKE VKNGFPKAHL IVANGMTDKK AQ\*

FIG.55

63/102

HOST	334	334	334	334	334	334	334	334	334	334	334
PLASMID	pYX242	pRAE-84	pYX242	pRAE-84	pYX242	pRAE-84	pYX242	pRAE-84	pYX242	pRAE-84	pYX242
SUBSTRATE	GLA	GLA	AA	AA	ADA	ADA	STA	STA	EPA	EPA	DPA
CONCENTRATION	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M
							% TOTAL LIPID				
C18:1n-9	15.94	14.16	12.30	15.67	11.77	11.41	14.81	17.92	15.91	16.33	15.04
C18:1n-7	1.25	1.21	1.10	1.50	1.13	1.18	1.19	1.38	1.33	1.49	1.37
C18:3n-6	4.53	4.21	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:4n-3	ND	ND	ND	ND	ND	ND	2.78	2.70	ND	ND	ND
C20:1n-7	ND	ND	ND	ND	ND	ND	0.32	ND	0.03	ND	0.05
C20:3n-6	0.10	0.37	ND	ND	ND	ND	ND	ND	0.05	ND	ND
C20:4n-6	ND	ND	11.44	5.55	ND	ND	ND	ND	ND	ND	ND
C20:4n-3	ND	ND	ND	ND	ND	ND	ND	ND	(14%)/0.44	ND	ND
C20:5n-3	ND	ND	ND	ND	ND	23.61	ND	ND	9.68	3.02	ND
C22:4n-6	ND	ND	(10.4%)/0.64	20.41	ND	ND	ND	ND	ND	ND	ND
C22:4n-3	ND	ND	ND	ND	ND	ND	(42.3%)/0.33	ND	ND	ND	ND
C22:5n-3	ND	ND	ND	ND	ND	ND	ND	ND	(32.7%)/1.47	ND	ND
C24:4n-6	ND	ND	(62.6%)/1.07	ND	(9.2%)/2.4	ND	ND	ND	ND	ND	ND
C24:5n-3	ND	ND	ND	ND	ND	ND	ND	(82.8%)/7.06	ND	ND	(43.9%)/3.82
TOTAL LIPID	208	126	115	189	158	149	124	433	221	271	127
											126

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

ND = NOT DETECTED

FIG. 56

64/102

HOST	334	334	334	334	334	334	334	334	334	334	334	334
PLASMID	pYY242	pRAE-84	pYY242	pRAE-84	pYY242	pRAE-84	pYY242	pRAE-84	pYY242	pRAE-84	pYY242	pRAE-84
SUBSTRATE	PA	PA	SA	SA	ARA	BA	BA	PTA	PA	PA	EA	EA
CONCENTRATION	25µM	25µM	25µM	25µM	% TOTAL LIPID	25µM	25µM	25µM	25µM	25µM	25µM	25µM
C16:0	36.30	39.85	7.12	8.31	5.78	4.42	4.17	5.76	18.69	18.85	14.69	15.25
C16:ln-7	26.22	23.52	11.77	15.25	10.23	6.29	7.01	10.10	38.48	41.23	20.55	31.48
C16:ln-5	0.23	0.28	0.16	0.20	0.13	0.07	0.09	0.13	0.38	0.26	0.43	0.35
C18:0	2.26	2.14	64.90	58.73	0.94	1.01	0.64	0.85	2.17	2.29	3.02	2.73
C18:ln-9	14.83	11.27	6.35	7.22	5.20	4.33	3.84	5.12	14.25	14.27	18.44	22.20
C18:ln-7	1.44	1.36	0.57	0.73	0.54	0.51	0.41	0.56	1.57	1.68	1.53	1.65
C18:ln-5	0.10	ND	0.06	ND	ND	ND	ND	0.06	0.17	0.15	ND	0.18
C20:0	0.59	0.24	0.09	0.08	66.40	74.78	0.10	0.05	0.17	0.17	0.24	0.20
C20:ln-9	0.06	0.10	ND	0.04	0.05	0.06	ND	ND	ND	ND	0.25	0.16
C20:ln-7	0.07	ND	ND	ND	0.12	ND	ND	ND	ND	ND	ND	ND
C22:0	0.45	0.75	0.29	0.30	0.43	0.31	77.35	70.71	0.74	0.80	0.98	0.74
C24:0	0.55	1.09	0.38	0.41	0.69	0.62	0.50	0.45	0.94	0.92	1.67	0.96
TOTAL LIPID	158	104	144	112	324	209	178	94	148	87	243	315
											70	529

SUBSTITUTE SHEET (RULE 26)

ND = NOT DETECTED

FIG.57A

65/102

HOST	334	334	334	334	334	334	334	334	334
PLASMID	pYX242	pRAE-84	pYX242	pRAE-84	pYX242	pRAE-84	pYX242	pRAE-84	pYX242
SUBSTRATE	LA	LA	GLA	GLA	DGLA	AA	AA	ADA	ADA
CONCENTRATION	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M
% TOTAL LIPID									
C18:1n-9	12.30	16.12	15.63	16.28	14.28	13.77	16.21	15.04	15.38
C18:1n-7	1.34	1.87	1.69	1.90	1.41	1.61	1.61	1.62	1.51
C18:2n-6	2.67	3.61	0.17	0.20	0.24	0.21	0.09	0.09	0.06
C18:3n-6	ND	ND	2.03	2.49	ND	ND	ND	ND	ND
C20:3n-6	ND	ND	ND	(14.7%)0.43	10.59	10.73	ND	ND	ND
C20:4n-6	ND	ND	ND	ND	ND	ND	14.03	5.27	ND
C22:4n-6	ND	ND	ND	ND	ND	ND	(8.7%)0.5	11.44	16.60
C24:0	0.79	1.00	1.08	1.16	1.30	0.87	0.87	0.72	0.77
C24:4n-6	ND	ND	ND	ND	ND	ND	(43.8%)0.39	ND	(7.3%)1.3
C24:5n-6	ND	ND	ND	ND	ND	ND	0.38	ND	ND
TOTAL LIPID	85	87	88	79	107	98	208	212	304
									122

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)  
ND = NOT DETECTED

FIG.57B

66/102

HOST	334	334	334	334	334	334	334	334	334
PLASMID	PYX242	PRAE-84	PYX242	PRAE-84	PYX242	PRAE-84	PYX242	PRAE-84	PYX242
SUBSTRATE	ALA	ALA	STA	STA	EPA	EPA	DPA	DPA	DPA
CONCENTRATION	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M	25 $\mu$ M
% TOTAL LIPID									
C18:ln-9	16.69	16.38	18.24	15.95	14.07	15.16	16.05	15.06	17.47
C18:ln-7	1.37	1.43	1.71	1.40	1.37	1.47	1.67	1.51	1.75
C18:ln-6	0.08	0.08	0.12	0.04	0.13	0.06	0.11	0.18	0.13
C18:3n-3	4.47	4.28	ND	ND	ND	ND	ND	ND	ND
C18:4n-3	ND	ND	2.28	2.39	ND	ND	ND	ND	ND
C20:3n-3	(1.3%)0.06	(3.6%)0.16	ND	ND	ND	0.26	ND	ND	ND
C20:4n-3	ND	ND	(11.1%)0.3	ND	ND	ND	ND	ND	ND
C20:5n-3	ND	0.07	ND	ND	9.97	3.84	ND	ND	ND
C22:4n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:5n-6	ND	ND	ND	(43.4%)0.23	ND	ND	0.64	0.55	ND
C22:5n-3	ND	ND	ND	ND	(24.0%)1.21	8.79	3.57	ND	ND
C24:0	0.65	0.43	1.41	0.58	1.38	0.78	1.45	1.35	0.89
C24:5n-3	ND	ND	ND	ND	(73.6%)3.38	ND	(46.4%)3.09	ND	ND
TOTAL LIPID	362	384	173	393	124	280	137	151	190
									200

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)  
ND = NOT DETECTED

FIG. 57C

67/102

1 ATGGAACATT TCGATGCGTC ACTCAGTACC TATTTCAGG CCTTCCTGG  
51 CCCCCGAGAT ACAAGAGTCA AAGGATGGTT CCTCCTGGAC AATTACATCC  
101 CTACGTTTGT CTGTTCTGTT ATTACTTAC TCATTGTATG GCTGGGACCA  
151 AAATACATGA AGAACCGGCA GCCGTTCTCT TGCCGAGGCA TCCTGCAGTT  
201 GTATAACCTT GGACTCACCC TGCTGTCTCT CTACATGTTC TATGAGTTGG  
251 TGACAGGTGT GTGGGAGGGC AAATACAAC TTTTCTGCCA GGGAACACGC  
301 AGCGCAGGGAG AATCCGATAT GAAGATCATC CGCGTCTCT GGTGGTACTA  
351 CTTCTCCAAA CTCATCGAAAT TCATGGACAC CTTTTCTTC ATCCTTCGCA  
401 AGAACAAACCA CCAGATCACC GTGCTCCATG TCTACCACCA CGCTACCATG  
451 CTCAACATCT GGTGGTTTGT GATGAACTGG GTTCCCTGCG GCCATTATA  
501 TTTTGGTGCG ACACTCAACA GCTTCATCCA TGTCTCATG TACTCGTACT  
551 ATGGTCTGTC CTCCATCCCG TCCATGCGTC CCTACCTCTG GTGGAAAAAG  
601 TACATCACTC AAGGGCAGCT GGTCCAGTTT GTGCTGACAA TCATCCAGAC  
651 GACCTGCGGG GTCTTCTGGC CATGCTCCTT CCCTCTCGGG TGGCTGTTCT  
701 TCCAGATTGG ATACATGATT TCCCTGATTG CTCTCTTCAC AAACTTCTAC  
751 ATTCAAGACTT ACAACAAGAA AGGGGCCTCT CGGAGGAAAG ACCACCTGAA  
801 GGGCCACCAAG AACGGGTCTG TGGCCGCCGT CAACGGACAC ACCAACAGCT  
851 TCCCTTCCCT GGAAAACAGC GTGAAGCCCA GGAAGCAGCG AAAGGATTGA

FIG.58

68/102

1 MEHFDASLST YFKAFLGPRD TRVKGFLLD NYIPTFVCSV IYLLIWLG  
51 KYMKNRQPFS CRGILQLYNL GLTLLSLYMF YELVTGVWEG KYNFFCQGTR  
101 SAGESDMKII RVLWWYYFSK LIEFMDTFFF ILRKNNHQIT VLHVYHHATM  
151 LNIWWFVMNW VPCGHSYFGA TLNSFIHVLM YSYYGLSSIP SMRPYLWWKK  
201 YITQGQLVQF VLTIIQTTCG VFWPCSFPLG WLFFQIGYMI SLIALFTNFY  
251 IQTYNKKGAS RRKDHLKGHQ NGSAVNGH TNSFPSLENS VKPRKQRKD\*

FIG.59

69/102

HOST	334	334	334	334	334	334	334	334	334	334	334	334
PLASMID	pYX242	pRAE-87	pYX242	pRAE-87	pYX242	pRAE-87	pYX242	pRAE-87	pYX242	pRAE-87	pYX242	pRAE-87
SUBSTRATE	GLA	AA	AA	ADA	ADA	ADA	STA	STA	EPA	EPA	DPA	DPA
CONCENTRATION	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM
% TOTAL LIPID												
C18:1n-9	15.94	12.05	12.30	12.61	11.77	10.91	14.81	15.52	15.91	16.66	15.04	8.07
C18:ln-7	1.25	8.00	1.10	9.60	1.13	8.87	1.19	8.94	1.33	11.60	1.37	6.90
C18:3n-6	4.53	1.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C18:4n-3	ND	ND	0.09	ND	0.14	2.78	0.80	ND	ND	ND	ND	ND
C20:1n-7	ND	0.98	ND	0.91	ND	0.63	ND	0.62	ND	0.94	ND	1.34
C20:3n-6	0.10	(78.7%)4.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:4n-6	ND	11.44	11.28	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:4n-3	ND	ND	ND	ND	ND	ND	ND	(81.0%)3.4	ND	ND	ND	ND
C20:5n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:4n-6	ND	ND	(36.0%)6.33	20.41	21.15	ND	ND	ND	9.68	4.58	ND	ND
C22:4n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C22:5n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.57	ND
C24:4n-6	ND	ND	ND	ND	ND	ND	ND	ND	(57.4%)6.18	7.87	17.24	ND
C24:5n-3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TOTAL LIPID	208	102	115	177	158	117	124	200	221	199	127	91

SUBSTITUTE SHEET (RULE 26)

(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

ND = NOT DETECTED

FIG. 60

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HOST(PLASMID)	334(pYX242)		334(pRET-22)		334(pYX242)		334(pRET-22)		334(pRET-22)	
	ADDED SUBSTRATES	50 mM GLA	50 mM GLA	50 mM AA	50 mM AA	NO SUBSTRATE				
% TOTAL LIPID										
FATTY ACID										
C16:0	19.8	18.59	13.8	6.23	13.62	13.63				
C16:1n-7	20.92	17.74	26.62	13.01	40.1	47.67				
C18:0	5.79	4.94	3.62	2	4.86	5.031				
C18:1n-7	(3.9%) 0.85	(9.12%) 1.78	(3.5%) 0.97	(12.54%) 1.18	(3.6%) 1.5	(7.53%) 3.88				
C18:1n-9	8.46	7.45	10.27	5.36	13.7	16.93				
C18:3n-6	*26.62	*22.03	0.03	0.01						
C20:3n-6	(1.1%) 0.3	(38.2%) 13.61								
C20:4n-6			*27.36	*65.38						
C22:4n-6										
TOTAL LIPID ( $\mu$ g)	36	42	85	280	55	79				
(% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)										
*INDICATES SUBSTRATE ADDED										

FIG. 61

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HOST(PLASMID)	334(pRET22)	334(pRET22)	334(pRET22)	334(pRET22)	334(pRET22)
ADDED SUBSTRATE	50µM SA	50µM OA	50µM LA	50µM DLGA	25µM AA
C18:0		C18:1n-9	C18:2n-6	C20:3n-6	C20:4n-6
% TOTAL LIPID					
FATTY ACID					
C16:0	12.9	12.54	15.23	9.1	10.2
C16:1	37.71	23.83	24.87	16.61	18.375
C18:0	11.44	4.7	4.49	2.7	2.9
C18:1n-9	14.03	*16.87	9.54	6.74	6.39
C18:2n-6			16.87		0.15
C18:3n-6					0.28
C20:2n-6					0.05
C20:3n-6				*44.34	
C20:4n-6				0.34	*25.78
C22:4n-6					*75.72
TOTAL LIPID (µg)	63	103	71	110	97
					277

FIG. 62A

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HOST(PLASMID)	334(pRET22)	334(pRET22)	334(pRET22)	334(pRET22)
ADDED SUBSTRATE	50 $\mu$ M ALA	50 $\mu$ M PA	50 $\mu$ M EPA	50 $\mu$ M STA
	C18:3n-3	C18:0	C20:5n-3	C18:4n-3
FATTY ACID				
C16:0	13.91	15.06	16.92	20.08
C16:1	14.74	31.77	23.57	20.17
C18:0	4.06	*4.85	4.94	6.02
C18:1n-9	6.65	13.59	10.46	9.29
C18:3n-3	*38.66			
C18:4n-3				
C20:4n-3				*20.45
C20:5n-3			(12.57%)	2.94
C22:5n-3			*15.48	
TOTAL LIPID ( $\mu$ g)	80	84	81	60

\*INDICATES SUBSTRATE ADDED  
 (% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

FIG. 62B

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HOST(PLASMID)	334(pRET-22+pCGR-4)	334(pYX242+pYES2)
ADDED SUBSTRATE	50 μM GLA	50 μM GLA
FATTY ACID	% TOTAL LIPID	
C16:0	15.92	15.07
C16:1n-7	24.97	19.48
C18:0	8.52	6.48
C18:1n-7	3.9	1.61
C18:1n-9	18.48	12.71
C18:3n-6	*7.0	*10.54
C20:0	0	0
C20:3n-6	(27.81%) 4.36	(1.58%) 0.17
C20:4n-6	(27.55%) 4.32	0
TOTAL LIPID (μg)	508	168

FIG.63A

HOST(PLASMID)	334(pRET-22+pCGR-4)	334(pYX242+pYES2)
ADDED SUBSTRATE	50 μM STA	50 μM STA
FATTY ACID	% TOTAL LIPID	
C16:0	18.74	16.21
C16:1n-7	21.35	26.09
C18:0	6.78	7.57
C18:1n-7	1.97	1.7
C18:1n-9	20.73	22.41
C18:4n-3	*6.05	*13.43
C20:0	0	0.45
C20:4n-3	(15.88%) 1.68	(4.73%) 0.69
C20:5n-3	(26.93%) 2.85	(3.22%) 0.47
TOTAL LIPID (μg)	335	161

\*INDICATES SUBSTRATE ADDED  
 (% CONVERSION)=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.63B

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FIG. 64A

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HSEL01	208	L Q F V L T I I Q T S C G V I W P -	C - -	- - T F P L G W L Y F Q I -	G Y M I S L I A	244
MEL04	211	V Q F V L T I T H T L S A V V K P -	C - -	- - G F P F G C L I F Q S -	S Y M M T L V I	247
GLELO	248	T Q F C M M S V Q S S W D M Y A M K		G R P G Y P F -	F I T A L L W F Y M W T M L G	289
CEELO	218	V Q F I I S C A V L A H L G Y L M H F T N A N C D F E P S V F K L A V F M D T T Y L A				260
HSEL01	245	L F T N F Y I Q T Y N K K G A S R R K D H L K D H Q N G S M A A V N G H T N S F S P L				287
MEL04	248	L F L N F Y I Q T Y R K K P V -	- - K K E L Q E - -	- - K - E V K N G F P K A H L I V		282
GLELO	290	L F Y N F - - - Y R K N A K L A K Q A K A D A A K E K A R K L Q				318
CEELO	261	L F V N F F Q L S Y V L R G G K D K Y K A V P K K N N				288
HSEL01	288	E N N V K P R K L R K D				299
MEL04	283	A N G M T D K K A Q				292

FIG. 64B

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1 ACAGGCGACT TTCTGTTGAA TTTGTTGCAG GCACAGCAAG GAAGGGATGGC  
51 AACAGCGAGC GTGTGGGATG ATGTGGTGGG CCGCGTGGAG ACCGGCGTGG  
101 ACCAGTGGAT GGATGGCGCC AAGCCGTACG CACTCACCGA TGGGCTCCG  
151 ATGATGGACG TGTCCACCAT GCTGGCATTG GAGGTGGGAT ACATGGCCAT  
201 GCTGCTCTTC GGCATCCCGA TCATGAAGCA GATGGAGAAG CCTTTTGAGC  
251 TCAAGACCAT CAAGCTCTTG CACAACTTGT TTCTCTTCGG ACTTTCTTG  
301 TACATGTGCG TGGAGACCAT CCGCCAGGCT ATCCTCGGAG GCTACAAAGT  
351 GTTTGGAAAC GACATGGAGA AGGGCAACGA GTCTCATGCT CAGGGCATGT  
401 CTCGCATCGT GTACGTGTT TACGTGTCCA AGGCATACGA GTTCTTGGAT  
451 ACCGCCATCA TGATCCTTG CAAGAAGTTC AACCAAGGTTT CCTTCTTGCA  
501 TGTGTACAC CATGCCACCA TTTTGCCAT CTGGTGGGCT ATGCCAAGT  
551 ACGCTCCAGG AGGTGATGCG TACTTTTCAG TGATCCTAAA CTCTTCATC  
601 CACGTTATCA TGTACTCTTA CTACCCCTGAA

FIG. 65

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1 TGDFLLNLLQ AQQGRMANSS VVDDVVGRVE TGVDQWMDGA KPYALTDLGP  
51 MMDVSTMLAF EVGYMAMLLF GIPIMKQMEK PFELKTIKLL HNLFLFGLSL  
101 YMCVETIRQA ILGGYKVFGN DMEKGNESHA QGMSRIVYVF YVSKAYEFLD  
151 TAIMILCKKF NQVSFLHVYH HATIFAIWWA IAKYAPGGDA YFSVILNSFI  
201 HVIMYSYYPE

## FIG.66

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1 ATGGCAAACA GCAGCGTGTG GGATGATGTG GTGGGCCGCG TGGAGACCGG  
51 CGTGGACCAG TGGATGGATG GCGCCAAGCC GTACGCACTC ACCGATGGC  
101 TCCCGATGAT GGACGTGTCC ACCATGCTGG CATTGAGGT GGGATACATG  
151 GCCATGCTGC TCTTCGGCAT CCCGATCATG AAGCAGATGG AGAAGCCTT  
201 TGAGCTCAAG ACCATCAAGC TCTTGACAA CTTGTTCTC TTCGGACTT  
251 CCTTGTACAT GTGCGTGGAG ACCATCCGCC AGGCTATCCT CGGAGGCTAC  
301 AAAGTGTGGAAACGACAT GGAGAAGGGC AACGAGTCTC ATGCTCAGGG  
351 CATGTCTCGC ATCGTGTACG TGTTCTGCGT GTCCAAGGCA TACGAGTTCT  
401 TGGATAACCGC CATCATGATC CTTTGCAAGA AGTTCAACCA GGTTTCCTTC  
451 TTGCAATGTGT ACCACCATGC CACCATTTT GCCATCTGGT GGGCTATCGC  
501 CAAGTACGCT CCAGGAGGTG ATGCGTACTT TTCAGTGATC CTCAACTCTT  
551 TCGTGCACAC CGTCATGTAC GCATACTACT TCTTCTCCTC CCAAGGGTTC  
601 GGGTTCGTGA AGCCAATCAA GCCGTACATC ACCACCCCTTC AGATGACCCA  
651 GTTCATGGCA ATGCTGTGC AGTCCTTGTG CGACTACCTC TTCCCCATGCG  
701 ACTACCCACA GGCTCTGTG CAGCTTCTTG GAGTGTACAT GATCACCTG  
751 CTTGCCCTCT TCGGCAACTT TTTTGTGCAAG AGCTATCTTA AAAAGCCAAA  
801 AAAGAGCAAG ACCAACTAA

FIG.67

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1 ATGGCAAACA GCAGCGTGTG GGATGATGTG GTGGGCCGCG TGGAGACCGG  
51 CGTGGACCAAG TGGATGGATG GCGCCAAGCC GTACGCACTC ACCGATGGC  
101 CCCCGATGAT GGACGTGTCC ACCATGCTGG CATTGAGGT GGGATACATG  
151 GCCATGCTGC TCTTCGGCAT CCCGATCATG AAGCAGATGG AGAAGCCTT  
201 TGAGCTCAAG ACCATCAAGC TCTTGACAAA CTTGTTCTC TTCGGACTT  
251 CCTTGATACAT GTGCGTGGAG ACCATCCGCC AGGCTATCCT CGGAGGCTAC  
301 AAAGTGTGG AAAACGACAT GGAGAAGGGC AACGAGTCTC ATGCTCAGGG  
351 CATGTCTCGC ATCGTGTACG CGTTCTACGT GTCCAAGGCA TACGAGTTCT  
401 TGGATAACCGC CATCATGATC CTTTGCAAGA AGTTCAACCA GGTTTCCTTC  
451 TTGCAATGT ACCACCATGC CACCATTGTT GCCATCTGGT GGGCTATCGC  
501 CAAGTACGCC CCAGGAGGTG ATGCGTACTT TTCAGTGATC CTCAAACCTT  
551 TCGTGCACAC CGTCATGTAC GCATACTACT TCTTCTCCTC CCAAGGGTTC  
601 GGGTTCGTGA AGCCAATCAA GCCGTACATC ACCACCCCTTC AGATGACCCA  
651 GTTCATGGCA ATGCTTGTGC AGTCCTTGTG CGACTACCTC TTCCCATGCG  
701 ACTACCCACA GGCTCTTGTG CAGCTTCTTG GAGTGTACAT GATCACCTG  
751 CTTGCCCTCT TCGGCAACTT TTTTGTGCAG AGCTATCTTA AAAAGCCAAA  
801 AAAGAGCAAG ACCAACTAA

FIG.68

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1 ATGGCAACAG CAGCGTGTGG GATGATGTGG TGGGCCGCGT GGAGACCAGC  
51 GTGGACCACT GGATGGATGG CGCCAAGCCG TACGCACCTCA CCGATGGGCT  
101 CCCGATGATG GACGTGTCCA CCATGCTGGC ATTGAGGTG GGATACATGG  
151 CCATGCTGCT CTTCGGCATC CCGATCATGA AGCAGATGGA GAAGCCTTT  
201 GAGCTCAAGA CCATCAAGCT CTTGCACAAAC TTGTTTCTCT TC GGACTTTC  
251 CTTGTACATG TGCGTGGAGA CCATCCGCCA GGCTATCCTC GGAGGCTACA  
301 AAGTGTGG AAACGACATG GAGAAGGGCA ACGAGTCTCA TGCTCAGGGC  
351 ATGTCTCGCA TCGTGTACGT GTTCTACGTG TCCAAGGCAT ACGAGTTCTT  
401 GGATACCGCC ATCATGATCC TTTGCAAGAA GTTCAACCAG GTTCCCTCT  
451 TGCAAGTGTA CCACCATGCC ACCATTITTG CCATCTGGTG GGCTATCGCC  
501 AAGTACGCTC CAGGAGGTGA TGCGTACTTT TCAGTGATCC TCAACTCTT  
551 CGTGCACACC GTCATGTACG CATACTACTT CTTCTCCTCC CAAGGGTTCG  
601 GGTTCGTCAA GCCAATCAAG CCGTACATCA CCACCCCTCA GATGACCCAG  
651 TTCATGGCAA TGCTTGTGCA GTCCTTGAC GACTACCTCT TCCCATGCAG  
701 CTACCCACAG GCTCTTGTGC AGCTTCTTGG AGTGTACATG ATCACCTTGC  
751 TTGCCCTCTT CGGCAACTTT TTTGTGCAGA GCTATCTAA AAAGCCAAA  
801 AAGAGCAAGA CCAACTAA

FIG.69

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1 ATGGCAAACA GCAGCGTGTG GGATGATGTG GTGGGCCGCG TGGAGACCGG  
51 CGTGGACCAG TGGATGGATG GCGCCAAGCC GTACGCACTC ACCGATGGC  
101 TCCCGATGAT GGACGTGTCC ACCATGCTGG CATTGAGGT GGGATAATG  
151 GCCATGCTGC TCTTCGGCAT CCCGATCATG AAGCAGATGG AGAAGCCTT  
201 TGAGCTCAAG ACCATCAAGC TCTTGACAA CTTGTTCTC TTCGGACTT  
251 CCTTGTACAT GTGCGTGGAG ACCATCCGCC AGGCTATCCT CGGAGGCTAC  
301 AAAGTGTGAA GAAACGACAT GGAGAAGGGC AACGAGTCTC ATGCTCAGGG  
351 CATGTCTCGC ATCGTGTACG TGTTCTACGT GTCCAAGGCA TACGAGTTCT  
401 TGGATACCGC CATCATGATC CTTGCAAGA AGTTCAACCA GGTTCCTTC  
451 TTGCATGTGT ACCACCATGC CACCGTTTT GCCATCTGGT GGGCTATCGC  
501 CAAGTACGCT CCAGGAGGTG ATGCGTACTT TTCAGTGATC CTCAACTCTT  
551 TCGTGCACAC CGTCATGTAC GCATACTACT TCTTCTCCTC CCAAGGGTTC  
601 GGGTTCGTGA AGCCAATCAA GCCGTACATC ACCACCCCTTC AGATGACCCA  
651 GTTCATGGCA ATGCTTGTGC AGTCCTTGTG CGACTACCTC TTCCCATGCG  
701 ACTACCCACA GGCTCTTGTG CAGCTTCTTG GAGTGTACAT GATCACCTTG  
751 CTTGCCCTCT TCGGCAACTT TTTTGTGCAG AGCTATCTTA AAAAGCCAAA  
801 AAAGAGCAAG ACCAACTAA

FIG.70

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1 ATGGCAAACA GCAGCGTGTG GGATGGTGTG GTGGGCCGCG TGGAGACCGG  
51 CGTGGACCAG TGGATGGATG GCGCCAAGCC GTACGCACTC ACCGATGGC  
101 TCCCAGATGAT GGACGTGTCC ACCATGCTGG CATTGAGGT GGGATACATG  
151 GCCATGCTGC TCTTCGGCAT CCCGATCATG AAGCAGATGG AGAAGCCTT  
201 TGAGCTCAAG ACCATCAAGC TCTTGACAA CTTGTTCTC TTCGGACTT  
251 CCTTGTACAT GTGCGTGGAG ACCATCCGCC AGGCTATCCT CGGAGGCTAC  
301 AAAGTGTGG GAAACGACAT GGAGAAGGGC AACGAGTCTC ATGCTCAGGG  
351 CATGTCTCGC ATCGTGTACG TGTTCTACGT GTCCAAGGCA TACGAGTTCT  
401 TGGATAACCGC CATCATGATC CTTTGCAAGA AGTTCAACCA GGTTTCTTC  
451 TTGCAATGCGT ACCACCATGC CACCATTTT GCCATCTGGT GGGCTATCGC  
501 CAAGTACGCT CCAGGAGGTG ATGCGTACTT TTCAGTGATC CTCAACTCTT  
551 TCGTGCACAC CGTCATGTAC GCATACTACT TCTTCTCCTC CCAAGGGTTC  
601 GGGTTCGTGA AGCCAATCAA GCCGTACATC ACCACCCCTTC AGATGACCCA  
651 GTTCATGGCA ATGCTTGTGC AGTCCTTGTG CGACTACCTC TTCCCCATGCG  
701 ACTACCCACA GGCTTCTGTG CAGCTTCTTG GAGTGTACAT GATCACCTG  
751 CTTGCCCTCT TCGGCAACTT TTTTGTGCAAG AGCTATCTTA AAAAGCCAAA  
801 AAAGAGCAAG ACCAACTAA

FIG. 71

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1 ATGGCAAACA GCAGCGTGTG GGATGATGTG GTGGGCCGCG TGGAGACCGG  
51 CGTGGACCAG TGGATGGATG GCGCCAAGCC GTACGCACTC ACCGATGGGC  
101 TCCCGATGAT GGACGTGTCC ACCATGCTGG CATTGAGGT GGGATACATG  
151 GCCATGCTGC TCTTCGGCAT CCCGATCATG AAGCAGATGG AGAAGCCTTT  
201 TGAGCTCAAG ACCATCAAGC TCTTGACAA CTTGTTCTC TTCGGACTTT  
251 CCTTGTACAT GTGCGTGGAG ACCATCCGCC AGGCTATCCT CGGAGGCTAC  
301 AAAGTTTG GAAACGACAT GGAGAAGGGC AACGAGTCTC ATGCTCAGGG  
351 CATGTCTCGC ATCGTGTACG TGTTCTACGT GTCCAAGGCA TACGAGTTCT  
401 TGGATAACCGC CATCATGATC CTTGCAAGA AGTTCAACCA GGTTTCTTC  
451 TTGCATGTGT ACCACCATGC CACCATTGTT GCCATCTGGT GGGCTATCGC  
501 CAAGTACGCT CCAGGAGGTG ATGCGTACTT TTCAGTGATC CTCAACTCTT  
551 TCGTGCACAC CGTCATGTAC GCATACTACT TCTTCTCCTC CCAAGGGTTC  
601 GGGTTCGTGA AGCCAATCAA GCCGTACATC ACCACCCCTTC AGATGACCCA  
651 GTTCATGGCA ATGCTGTGC AGTCCTTGTG CGACTACCTC TTCCCATGCG  
701 ACTACCCACA GGCTCTGTG CAGCTCCTTG GAGTGTACAT GATCACCTTG  
751 CTTGCCCTCT TCAGCAACTT TTTGTGCAG AGCTATCTTA AAAAGCCAAA  
801 AAAGAGCAAG ACCAACTAA

FIG.72

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1 ATGGCAAACA GCAGCGTGTG GGATGATGTG GTGGGCCGCG TGGAGACCGG  
51 CGTGGACCAG TGGATGGATG GCGCCAAGCC GTACGCACTC ACCGATGGGC  
101 TCCCGATGAT GGACGTGTCC ACCATGCTGG CATTGAGGT GGGATAACATG  
151 GCCATGCTGC TCTTCGGCAT CCCGATCATG AGGCAGATGG AGAAGCCTTT  
201 TGAGCTCAAG ACCATCAAGC TCTTGACAAA CTTGTTCTC TTGGACTTT  
251 CCTTGTACAT GTGCGTGGTG ACCATCCGCC AGGCTATCCT TGGAGGCTAC  
301 AAAGTGTGG AAAACGACAT GGAGAAGGGC AACGAGTCTC ATGCTCAGGG  
351 CATGTCTCGC ATCGTGTACG TGTTCTACGT GTCCAAGGCÀ TACGAGTTCT  
401 TGGATAACCGC CATCATGATC CTTTGCAAGA AGTTCAACCA GGTTTCCCTTC  
451 TTGCATGTGT ACCACCATGC CACCATTTC GCCATCTGGT GGGCTATCGC  
501 CAAGTACGCT CCAGGAGGTG ATGCGTACTT TTCAGTGATC CTCAACTCTT  
551 TCGTGCACAC CGTCATGTAC GCATACTACT TCTTCTCCTC CCAAGGGTTC  
601 GGGTTCGTGA AGCCAATCAA GCCGTACATC ACCACCCCTTC AGATGACCCA  
651 GTTCATGGCA ATGCTTGTGC AGTCCTTGTG CGACTACCTC TTCCCCATGCG  
701 ACTACCCACA GGCTCTTGTG CAGCTTCTTG GAGTGTACAT GATCACCTTG  
751 CTTGCCCTCT TCGGCAACTT TTTTGTGCAG AGCTATCTTA AAAAGCCAAA  
801 AAAGAGCAAG ACCAACTAA

## FIG.73

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1 MANSSVWDDV VGRVETGVDQ WMDGAKPYAL TDGLPMMDVS TMLAFEVGYM  
51 AMLLFGIPIM KQMEKPFELK TIKLLHNLF<sub>L</sub> FGLSLYMCVE TIRQAILGGY  
101 KVFGNDMEKG NESHAQGMSR IVYVFCVSKA YEFLDTAIMI LCKKFNQVSF  
151 LHVYHHATIF AIWWAIAKYA PGGDAYFSVI LNSFWHTVMY AYYFFSSQGF  
201 GFVKPIKPYI TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMITL  
251 LALFGNFFVQ SYLKKPKKSK TN\*

FIG.74

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1 MANSSVWDDV VGRVETGVDQ WMDGAKPYAL TDGPPMMDVS TMLAFEVGYM  
51 AMLLFGIPIM KQMEKPFELK TIKLLHNLFL FGLSLYMCVE TIRQAILGGY  
101 KVFGNDMEKG NESHAQGMSR IVYAFYVSKA YEFLDTAIMI LCKKFNQVSF  
151 LHVYHHATIF AIWWAIAKYA PGGDAYFSVI LNSFVHTVMY AYYFFSSQGF  
201 GFVKPIKPYI TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMITL  
251 LALFGNFFVQ SYLKKPKKSK TN\*

FIG.75

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1 MATAACGMMW WAAWRPAWTS GWMAPSRTHS PMGSR\*WTCP. PCWHSRWDTW  
51 PCCSSASRS\* SRWRSLLSSR PSSSCTTCFS SDFPCTCAWR PSARLSSEAT  
101 KCLETTWRRA TSLMLRACLA SCTCSTCPRH TSSWIIPPS\*S FARSSTRFPS  
151 CKCTTMPPFL PSGGLSPSTL QEVMRTFQ\*S STLSCTPSCT HTTSSPPKGS  
201 GS\*SQSSRTS PPFR\*PSSWQ CLCSPCTTS SHATTHRLLC SFLECT\*SPC  
251 LPSSATFLCR AILKSQKRAR PT

FIG.76

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1 MANSSVWDDV VGRVETGVDQ WMDGAKPYAL TDGLPMMDVS TMLAFEVGYM  
51 AMLLFGIPIM KQMEKPFLK TIKLLHNLFL FGLSLYMCVE TIRQAILGGY  
101 KVFGNDMEKG NESHAQGMSR IVYVFYVSKA YEFLDTAIMI LCKKFNQVSF  
151 LHVVHHATVF AIWWAIAKYA PGGDAYFSVI LNSFVHTVMY AYYFFSSQGF  
201 GFVKPIKPYI TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMITL  
251 LALFGNFFVQ SYLKKPKKSK TN\*

FIG.77

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1 MANSSVWDGV VGRVETGVDQ WMDGAKPYAL TDGLPMMDVS TMLADEVGYM  
51 AMLLFGIPIM KQMEKPFELK TIKLLHNLFL FGLSLYMCVE TIRQAILGGY  
101 KVFGNDMEKG NESHAQGMSR IVYVFYVSKA YEFLDTAIMI LCKKFNQVSF  
151 LHAYHHATIF AIWWAIAKYA PGGDAYFSVI LNSFVHTVMY AYYFFSSQGF  
201 GFVKPIKPYI TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMITL  
251 LALFGNFFVQ SYLKKPKKSK TN\*

FIG.78

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1 MANSSVWDDV VGRVETGVDQ WMDGAKPYAL TDGLPMMDVS TMLADEVGYM  
51 AMLLFGIPIM KQMEKPFEKL TIKLLHNLF L FGLSLYMCVE TIRQAILGGY  
101 KVFGNDMEKG NESHAQGMSR IVYVFYVSKA YEFLDTAIMI LCKKFNQVSF  
151 LHVYHHATIF AIWWAIAKYA PGGDAYFSVI LNSFVHTVMY AYYFFSSQGF  
201 GFVKPIKPYI TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMITL  
251 LALFGNFFVQ SYLKKPKKSK TN\*

FIG.79

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1 MANSSVWDDV VGRVETGVDQ WMDGAKPYAL TDGLPMMDVS TMLAFEVGYM  
51 AMLLFGIPIM RQMEKPFEKL TIKLLHNLFK FGLSLYMCVV TIRQAILGGY  
101 KVFGNDMEKG NESHAQGMSR IVYVFYVSKA YEFLDTAIMI LCKKFNQVSF  
151 LHVYHHATIF AIWWAIAKYA PGGDAYFSVI LNSFVHTVMY AYYFFSSQGF  
201 GFVKPIKPYI TTLQMTQFMA MLVQSLYDYL FPCDYPQALV QLLGVYMITL  
251 LALFGNFFVQ SYLKKPKKSK TN\*

FIG.80

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334(PLASMID)	prAT-4-A1	prAT-4-A2	prAT-4-A3	prAT-4-A4	prAT-4-A6	prAT-4-A7	prAT-4-D1	pYX242
	GLA	GLA						
% TOTAL LIPID								
C16:1n-7	16.95	5.22	15.52	11.06	14.49	15.69	15.18	11.24
C18:0	4.95	6.73	5.94	4.17	4.57	4.25	4.79	5.64
C18:1n-9	20.26	44.21	22.54	17.22	16.89	16.51	18.92	28.72
C18:1n-7	2.87	1.64	2.97	7.51	1.85	3.90	3.50	1.70
C18:2n-6	1.03	13.79	1.44	0.67	1.32	0.28	0.36	5.53
C18:3n-6	3.05	1.09	5.28	2.38	1.89	2.45	2.79	2.24
C20:3n-6	10.09	0.43	0.21	11.04	5.76	10.77	9.28	
C22:0		0.57						
<hr/>								
% CONVERSION*	76.8%	28.4%	3.8%	82.2%	75.3%	81.5%	76.9%	

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.81A

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ADDED SUBSTRATE	EPA	EPA	EPA	EPA	% TOTAL LIPID		pYX242	
					PRAT-4-A1	PRAT-4-A2	PRAT-4-A3	PRAT-4-A4
C16:ln-7	18.35	17.65	7.96	11.80	14.06	11.53	9.68	20.66
C18:0	3.41	3.69	2.57	2.90	3.18	3.10	3.20	8.49
C18:ln-9	15.22	16.44	10.69	12.28	13.01	11.50	12.85	16.27
C18:ln-7	1.36	2.12	1.60	5.62	2.13	2.02	2.35	1.33
C18:2n-6	1.07	0.40			0.17		0.21	0.83
C20:5n-3	23.24	31.54	46.96	37.98	40.40	32.68	43.67	13.55
C22:ln-9		0.09	0.12	0.09				
C22:5n-3	0.40	0.16		3.13	1.37	4.61	1.27	
% CONVERSION*	1.7%	0.5%		7.6%	3.3%	12.4%	2.8%	

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.81B

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PRAT-4-A1	1	M A N S S V W D D V V G R V E T G V D Q W M D G A K P Y A L T D G L P M M D V S T M L A F E V	47
PRAT-4-A2	1	M A N S S S V W D D V V G R V E T G V D Q W M D G A K P Y A L T D G P P M M D V S T M L A F E V	47
PRAT-4-A4	1	M A N S S S V W D D V V G R V E T G V D Q W M D G A K P Y A L T D G L P M M D V S T M L A F E V	47
PRAT-4-A6	1	M A N S S S V W D G V V G R V E T G V D Q W M D G A K P Y A L T D G L P M M D V S T M L A F E V	47
PRAT-4-A7	1	M A N S S S V W D D V V G R V E T G V D Q W M D G A K P Y A L T D G L P M M D V S T M L A F E V	47
PRAT-4-D1	1	M A N S S S V W D D V V G R V E T G V D Q W M D G A K P Y A L T D G L P M M D V S T M L A F E V	47
PRAT-4-A1	48	G Y M A M L L F G I P I M K Q M E K P F E L K T I K L L H N L F L F G L S L Y M C V E T I R Q	94
PRAT-4-A2	48	G Y M A M L L F G I P I M K Q M E K P F E L K T I K L L H N L F L F G L S L Y M C V E T I R Q	94
PRAT-4-A4	48	G Y M A M L L F G I P I M K Q M E K P F E L K T I K L L H N L F L F G L S L Y M C V E T I R Q	94
PRAT-4-A6	48	G Y M A M L L F G I P I M K Q M E K P F E L K T I K L L H N L F L F G L S L Y M C V E T I R Q	94
PRAT-4-A7	48	G Y M A M L L F G I P I M K Q M E K P F E L K T I K L L H N L F L F G L S L Y M C V E T I R Q	94
PRAT-4-D1	48	G Y M A M L L F G I P I M K Q M E K P F E L K T I K L L H N L F L F G L S L Y M C V E T I R Q	94
PRAT-4-A1	95	A I L G G Y K V F F G N D M E K G N E S H A Q G M S R I V Y V F C V S S K A Y E F L D T A I M I L	141
PRAT-4-A2	95	A I L G G Y K V F F G N D M E K G N E S H A Q G M S R I V Y V F C V S S K A Y E F L D T A I M I L	141
PRAT-4-A4	95	A I L G G Y K V F F G N D M E K G N E S H A Q G M S R I V Y V F C V S S K A Y E F L D T A I M I L	141
PRAT-4-A6	95	A I L G G Y K V F F G N D M E K G N E S H A Q G M S R I V Y V F C V S S K A Y E F L D T A I M I L	141
PRAT-4-A7	95	A I L G G Y K V F F G N D M E K G N E S H A Q G M S R I V Y V F C V S S K A Y E F L D T A I M I L	141
PRAT-4-D1	95	A I L G G Y K V F F G N D M E K G N E S H A Q G M S R I V Y V F C V S S K A Y E F L D T A I M I L	141
PRAT-4-A1	142	C K K F N Q V S F L H V V H A T I F A I W W A I A K Y A P G G D A Y F S V I L N S F V H T V	188
PRAT-4-A2	142	C K K F N Q V S F L H V V H A T I F A I W W A I A K Y A P G G D A Y F S V I L N S F V H T V	188
PRAT-4-A4	142	C K K F N Q V S F L H V V H A T V F A I W W A I A K Y A P G G D A Y F S V I L N S F V H T V	188
PRAT-4-A6	142	C K K F N Q V S F L H V V H A T I F A I W W A I A K Y A P G G D A Y F S V I L N S F V H T V	188
PRAT-4-A7	142	C K K F N Q V S F L H V V H A T I F A I W W A I A K Y A P G G D A Y F S V I L N S F V H T V	188
PRAT-4-D1	142	C K K F N Q V S F L H V V H A T I F A I W W A I A K Y A P G G D A Y F S V I L N S F V H T V	188

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PRAT-4-A1	189	M Y A Y Y F F S S Q G F G F V K P I K P Y I T T L Q M T Q F M A M L V Q S L Y D Y L F P C D Y	235
PRAT-4-A2	189	M Y A Y Y F F S S Q G F G F V K P I K P Y I T T L Q M T Q F M A M L V Q S L Y D Y L F P C D Y	235
PRAT-4-A4	189	M Y A Y Y F F S S Q G F G F V K P I K P Y I T T L Q M T Q F M A M L V Q S L Y D Y L F P C D Y	235
PRAT-4-A6	189	M Y A Y Y F F S S Q G F G F V K P I K P Y I T T L Q M T Q F M A M L V Q S L Y D Y L F P C D Y	235
PRAT-4-A7	189	M Y A Y Y F F S S Q G F G F V K P I K P Y I T T L Q M T Q F M A M L V Q S L Y D Y L F P C D Y	235
PRAT-4-D1	189	M Y A Y Y F F S S Q G F G F V K P I K P Y I T T L Q M T Q F M A M L V Q S L Y D Y L F P C D Y	235
PRAT-4-A1	236	P Q A L V Q L L G V Y M I T L L A L F G N F F V Q S Y L K K P K K S K T N	272
PRAT-4-A2	236	P Q A L V Q L L G V Y M I T L L A L F G N F F V Q S Y L K K P K K S K T N	272
PRAT-4-A4	236	P Q A L V Q L L G V Y M I T L L A L F G N F F V Q S Y L K K P K K S K T N	272
PRAT-4-A6	236	P Q A L V Q L L G V Y M I T L L A L F G N F F V Q S Y L K K P K K S K T N	272
PRAT-4-A7	236	P Q A L V Q L L G V Y M I T L L A L F G N F F V Q S Y L K K P K K S K T N	272
PRAT-4-D1	236	P Q A L V Q L L G V Y M I T L L A L F G N F F V Q S Y L K K P K K S K T N	272

FIG.82B

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SCORES Init1: 259 Initn: 259 Opt: 509 z-score: 86.5 E(): 0.28  
>>EN:HSEL01 (300 aa)

initn: 259 initl: 259 opt: 509 Z-score: 86.5 expect(): 0.28

Smith-Waterman score: 511; 34.0% identity in 265 aa overlap  
(4-265:9-257)

	10	20	30	40	50	
TEL01	MANSSVWDDVVGRVETGVDQWMDGAKPYALTDLPMMDVSTMLA <b>F</b> EVGYMAMLLF					
HSEL01	MEHFDASLSTYFKALLGPRDTRVKGW-----FLLDNYIPTFICSVI-----YLLIW <b>L</b>					
	10	20	30	40		
	60	70	80	90	100	110
TEL01	GIPIMKQMEKP <b>F</b> EKLTKLLHNLFGLSLYMCVETIRQAILGGYKVFGNDMEKGNESHA					
HSEL01	:: ::   :   ::             : :    :   : : ::					
	GPKYMRN-KOPFSCRGILVVYNLGLTLLSLYMFCELVTGVWEGKYNNFCQGTRAGESDM					
	50	60	70	80	90	100
	120	130	140	150	160	170
TEL01	QGMSRIVYVFYVSKAYEFLDTAIMILCKKFNQVSFLHVYHHATIFAIWWAIAKYAPGGDA					
HSEL01	: :  :: :       :   :    : :  :        :   :     : ::    :					
	K-IIRVLWWYYFSKLEFMDTFFFILRKNNHQITVLHVYHHASMLNIWWFVMNWVPCGHS					
	110	120	130	140	150	160
	180	190	200	210	220	230
TEL01	YFSVILNSFVHTVMYAYYFFSSQGFGFVKPI---KPYITTLQMTQFMAMLVQSLYDYLFP					
HSEL01	::      :  :     :   ::         :   : ::  :					
	YFGATLNSFIHVLMYSYYGLSS--VPSMRPYLWWKKYITQGQLLQFVLTIQTSCGVIWP					
	170	180	190	200	210	220
	240	250	260	270	280	290
TEL01	CDYPQALVQLLGVYM <b>I</b> TLLALFGNFFVQSYLKKPKKSKTNXNCLHDMPLAGVRIDSESEL					
HSEL01	:  : :      :  :    :: :					
	CTFPLGWLYFQIGYMI <b>S</b> LIALFTNFYIQTYNKK <b>G</b> ASRRKDHLKDHQNGSMAAVNGHTNSF					
	230	240	250	260	270	280

FIG. 83

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SCORES Init1: 278 Initn: 278 Opt: 515 z-score: 86.1 E(): 0.3  
 >EN:MELO4 (293 aa)

initn: 278 init1: 278 opt: 515 Z-score: 86.1 expect(): 0.3  
 Smith-Waterman score: 517; 34.1% identity in 267 aa overlap  
 (8-270:16-265)

	10	20	30	40	50	
TEL01	MANSSVWDDVVGRVETGVVDQWMGAKPYALTDGLPMMDVSTMLA FEVG YMA M					
	::   ::     . : * :    . : : :   :					
MELO4	MEQLKAFDNEVNAFLDNMFGRDSRVRGW-----FLLDSYLP-----FILTITYLLS					
	10	20	30	40		
	60	70	80	90	100	110
TEL01	LLFGIPIMKQMEKP-FELKTIKLLLHNLFGLSLYMCVETIRQAILGGYKVFGNDMEKGN					
	: :     : :   :     :            :        : : : : :: :: :: :					
MELO4	IWLGKYMKN--RPALSLRGILTLYNLAITLLSAYMLVELILSSWEGGYNLQCQNLD SAG					
	50	60	70	80	90	100
	120	130	140	150	160	170
TEL01	ESHAQGMSRIVYVFYVSKAYEFLDTAIMILCKKFNQVSFLHVYHHATIFAIWWAI AKYAP					
	: . : : : :            :                  :       : :					
MELO4	EGDVR-VAKVLWWYYFSKLVEFLDTIFFVLRKKTNQITFLHVYHASM FNIWWCVLN WIP					
	110	120	130	140	150	160
	180	190	200	210	220	
TEL01	GGDAYFSVILNSFVHTVMYAYYFFSSQGFGFKPI---KPYITTLQMTQFMAMLV QSLYD					
	: :   :     :   :   :     :         :   :   : :   : : :					
MELO4	CGQSFFGPTLNSFIHILMYSYYGLSV--FPSMHKYLWKKYLTQAQLVQFVLTITHLS A					
	170	180	190	200	210	220
	230	240	250	260	270	280
TEL01	YLFP CDYPQALVQLLG VYMITLLA LGFNFFVQSYLKPKKS KTNCLHD MPLAG VRIDS					
	:     :   : : :     :       :         :         :					
MELO4	VVKPCGFPFGCLI FQSSYMMTLVILFLNFYI QT YRK KPVK KELQEKEVKGFPKAHL IVA					
	230	240	250	260	270	280

FIG.84

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SCORES Init1: 412 Initn: 541 Opt: 628 z-score: 102.7 E(): 0.035  
 >>EN:GLELO (319 aa)

initn: 541 init1: 412 opt: 628 Z-score: 102.7 expect(): 0.035

Smith-Waterman score: 628; 43.4% identity in 244 aa overlap  
 (34-272:69-308)

	10	20	30	40	50	60
TEL01	SSVDDVVGRVETGVVDQWMGAKPYALTDLPMMDVSTMLA <b>F</b> EVGYMAMLLFGIPIMKQM					
	40	50	60	70	80	90
GLELO	LVAQAEKYIPTIVHHTRGFLVAVESPLARELPLMNPFHVLLIVLAYLVTVFVGMQIMKNF					
	70	80	90	100	110	120
TEL01	EKPFE <b>L</b> KTIKLLHNLFGLSLYMCVETIRQAILGGYKVFGNDMEKGNESHAQGMSRIVY					
	100	110	120	130	140	150
GLELO	ER-FEVKTFSLLNFC <b>L</b> VSISAYMCGGILYEAYQANYGLFENAAD--HTFKGLPMAKMIW					
	130	140	150	160	170	180
TEL01	VFYVSKAYEFLDTAIMILCKKFNQVFSFLHVYHHATIFAIWWAI <b>A</b> KYAPGGDAYFSVILNS					
	160	170	180	190	200	210
GLELO	LFYFSKIMEFVDTMIMVLKKNNRQISFLHVYHHSSIFTIWWLVTFVAPNGEAYFSAALNS					
	190	200	210	220	230	
TEL01	FVHTVMYAYYFFSSQGFGFVKPIKPYITTLQMTQFMAMLVQSLYD-YLFPC---DYPQA					
	220	230	240	250	260	270
GLELO	FIHVIMGYYFLSALGFQVSFIKFYITRSQMTQFCMMSVQSSWD <b>M</b> YAMKVLGRPGYPFF					
	240	250	260	270	280	290
TEL01	LVQLLGVYMITLLALFGNFFVQS <del>Y</del> LKKPKKS <del>K</del> TNXNCLHDMPLAGVRIDSESELRRHANS					
	280	290	300	310		
GLELO	ITALLWFYMWTMLGLFYNFYRKNA-KLA <b>K</b> QAKADA <b>A</b> KEKARKLQ					

FIG.85

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SCORES Init1: 94 Initn: 220 Opt: 302 z-score: 61.6 E(): 6.5  
 >>EN:CEELO (288 aa)

initn: 220 init1: 94 opt: 302 Z-score: 61.6 expect(): 6.5  
 Smith-Waterman score: 312; 34.3% identity in 239 aa overlap  
 (49-270:54-278)

	20	30	40	50	60	70	
TEL01	DQWMDGAKPYALTDLPMMDVSTMLAFLFEGYMAMILLFGIPIMKQMEKPFLKTIKL-LHN						
					::   . : : :    :        :		
CEELO	ATHGPKNFPDAEGRKFFADHFDVTIQASILYM-VVVFGTKWMRNRQPFQL-TIPLNIWN						
	30	40	50	.	60	70	80
	80	90		100	110	120	
TEL01	LFLFGLSLYMCVE-----TI-RQAILGGY-KVFGNDMEKGNEHAQGMSRIVYVFYVS						
	::  ::  :   :   :   ::  ::          :     :   :   :   :						
CEELO	FILAFAFSIAGAVKMTPEFFGTIANKGIVASYCKVF--DFTKGENGYW-----VWLFMAS						
	90	100	110		120		130
	130	140	150	160	170	180	
TEL01	KAYEFLDTAIMILCKKFNQVSFLHVYHHATIFAIWWAIAKYAPGGDAYFSVILNSFVHTV						
	:  ::    :  :  :  :  :            :  :    :        :						
CEELO	KLFELEVDTIFLVLRK--PLMFLHWYHHILTMIAWYSHPLTPGFNRY-GIYLNFVVHAF						
	140	150	160	170	180	190	
	190	200	210	220	230	240	
TEL01	MYAYYFFSSQGFGFKPIKYITTLQMTQFM---AMLVQSLYDYLFP---CDYPQALVQL						
	::  ::  :  :  :            :  :  :  :  :  :  :  :  :  :						
CEELO	MYSYYFLRSMKIRVPGFIAQAITSLQIVQFIISCAVL AHLGYLMHFTNANCDFEPSVFKL						
	200	210	220	230	240	250	
	250	260	270	280	290	300	
TEL01	LGVYM-ITLLALFGNFFVQSYLKPKKSKTNXNCLHDMPLAGVRIDSESELRRHANSIFF						
	:  :              :  :  :  :  :  :						
CEELO	-AVFMDTTYLALFVNFFLQSYVLRGKGDKYKAVPKKKNN						
	260	270	280				

## FIG.86

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ADDED SUBSTRATE	GLA	% TOTAL LIPID										STA	STA	STA	
		PRAT-4-A4	PRAT-4-A6	PRAT-4-A7	PRAT-4-D1	pXX242	PRAT-4-A4	PRAT-4-A6	PRAT-4-A7	pXX242	PRAT-4-A4				
C16:1n-7	36.29	39.18	38.58	38.84	45.68	30.36	35.19	33.47	33.70	45.06	34.43	39.63	38.03	38.74	45.94
C18:0	2.78	3.13	2.73	3.05	1.76	2.51	2.78	2.56	2.72	1.65	4.01	3.13	3.06	3.76	1.82
C18:1n-9	15.89	16.55	15.92	16.34	13.23	14.60	15.14	15.39	15.26	13.01	16.05	16.51	17.37	19.07	14.10
C18:1w7	5.91	2.01	3.44	2.06	0.93	5.72	1.94	3.75	2.22	1.03	4.92	1.78	3.78	2.52	0.95
C18:3n6	2.10	1.96	1.60	2.19	5.29										
C18:4w3											0.83	0.88	0.53	1.09	3.42
C20:3n6	4.56	4.23	5.42	3.88							0.04				
C20:4w6						16.53	13.61	14.21	17.13	7.54					
C20:4w3											2.27	3.25	3.95	4.01	0.06
C22:4w6							0.57	0.20	0.75	0.18					
C22:4n-3											1.10	0.19	0.63	0.16	
% CONVERSION* OF C18	68.4%	68.4%	77.2%	64.0%							80.3%	79.6%	89.7%	79.3%	
% CONVERSION* OF C20							3.4%	1.5%	5.0%	1.0%	32.6%	5.5%	13.8%	3.8%	

SUBSTITUTE SHEET (RULE 26)

FIG.87A

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334(PLASMID)	PRAT-4-A4	PRAT-4-A6	PRAT-4-A7	PRAT-4-D1	PRAT-4-A4	PRAT-4-A6	PRAT-4-A7	pRX242
ADDED SUBSTRATE	EPA	EPA	EPA	EPA	EPA	NONE	NONE	NONE
% TOTAL LIPID								
C16:1w7	31.51	33.92	34.31	34.74	47.12	39.54	41.51	41.44
C18:0	2.76	3.09	2.78	2.77	1.79	2.45	3.21	2.59
C18:1w9	15.69	15.79	16.49	15.22	13.95	18.56	19.08	17.98
C18:1w7	6.07	1.97	3.72	1.89	1.05	8.77	2.57	4.86
C20:1w7	0.22	0.08	0.13	0.07		0.61	0.07	0.14
C20:5w3	11.88	15.20	11.32	13.91	6.41			
C22:5n-3	1.49	0.54	1.74	0.54				
% CONVERSION*	11.1%		3.4%		13.3%		3.7%	

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

**SUBSTITUTE SHEET (RULE 26)**

FIG. 87B

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ADDED SUBSTRATE	IA	IAA	AlA	GLA	STA	STA	DGA	DGA	EIA	EIA	AA	AA	pYX242							
	% TOTAL LIPID																			
C16:1n-7	38.73	27.71	39.05	26.97	35.28	30.13	34.75	30.95	35.96	28.51	34.91	31.45	34.71	24.76	40.02	24.75				
C16:1n-5	0.37	0.17	0.32	0.20	0.25	0.21	0.27	0.18	0.33	0.22	0.31	0.21	0.32	0.20	0.40	0.21				
C18:0	2.21	2.80	2.14	3.27	2.12	3.14	2.44	3.28	2.15	2.86	2.55	3.52	2.42	2.71	2.75	3.26				
C18:1n-9	11.14	12.79	11.73	15.73	10.14	14.24	11.13	14.79	11.17	14.59	11.33	16.32	11.63	13.85	12.86	15.99				
C18:1n-7	0.85	3.19	0.73	3.84	0.64	3.31	0.78	3.01	0.96	3.71	0.95	3.89	1.06	3.49	1.11	3.87				
C18:2n-6	3.04	3.54																		
C18:3n-6																				
C18:3n-3																				
C18:4n-3																				
C20:1n-7	0.10	0.09																		
C20:2n-6		1.07																		
C20:3n-6																				
C20:4n-6																				
C20:3n-3																				
C20:4n-3																				
C20:5n-3																				
C22:4n-7		0.11	0.19	0.14	0.18	0.13	0.14	0.13	0.24	0.14	0.22	0.14	0.22	0.14	0.27					
C22:4n-6																	0.76			
C22:4n-3																	1.04			
C22:5n-3																			1.99	
% CONVERSION* OF C18	23.2%		43.3%		78.6%		85.3%													
% CONVERSION OF C20																				

SUBSTITUTE SHEET (RULE 26)

\*% CONVERSION=PRODUCT/(SUBSTRATE+PRODUCT)

FIG.88

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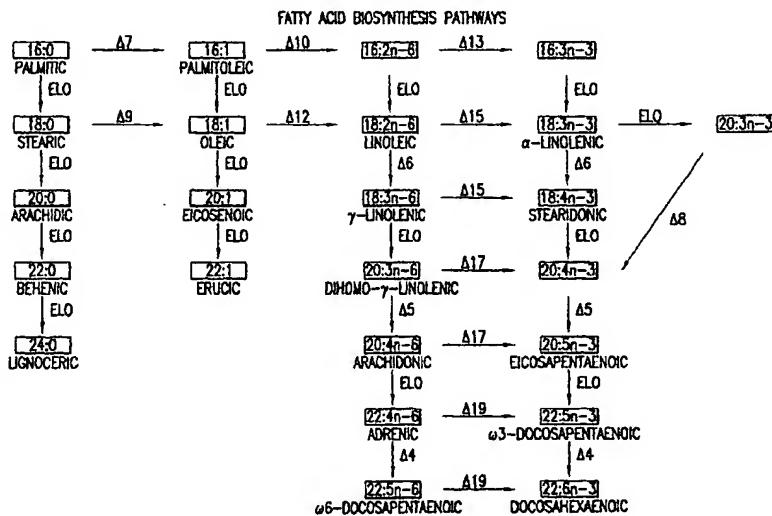
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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

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*[Continued on next page]*

(54) Title: ELONGASE GENES AND USES THEREOF



(57) Abstract: The subject invention relates to the identification of several genes involved in the elongation of polyunsaturated acids (i.e., "elongases") and to uses thereof. At least two of these genes are also involved in the elongation of monounsaturated fatty acids. In particular, elongase is utilized in the conversion of gamma linolenic acid (GLA) to dihomogamma linolenic acid (DGLA) and in the conversion of DGLA or 20:4n-3 to eicosapentaenoic acid (EPA). DGLA may be utilized in the production of polyunsaturated fatty acids, such as arachidonic acid (AA), docosahexaenoic acid (DHA), EPA, adrenic acid, w6-docosapentaenoic acid or w3-docosapentaenoic acid which may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

WO 02/008401 A3



IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,  
TG).

(88) Date of publication of the international search report:  
13 March 2003

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## INTERNATIONAL SEARCH REPORT

In  National Application No  
PCT/US 01/23259

A. CLASSIFICATION OF SUBJECT MATTER				
IPC 7	C12N15/54	C12N9/10	C12N1/15	C12N1/19
	C12N5/10	A01K67/027	A23D9/00	A23L1/29
	A61K7/00			A61K31/20

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01K A23D A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TVRDIK P ET AL: "ROLE OF A NEW MAMMALIAN GENE FAMILY IN THE BIOSYNTHESIS OF VERY LONG CHAIN FATTY ACIDS AND SPHINGOLIPIDS" THE JOURNAL OF CELL BIOLOGY, ROCKEFELLER UNIVERSITY PRESS, US, vol. 149, no. 3, 1 May 2000 (2000-05-01), pages 707-717, XP000986889 ISSN: 0021-9525 the whole document ---	1-24, 26-34
Y	---	8-14,48
X,L	WO 00 12720 A (ABBOTT LAB) 9 March 2000 (2000-03-09) cited in the application	25,47, 49,50, 52, 54-62,73
Y	L: priority. the whole document ---	48,51, 53, 63-72, 74-82
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

2 December 2002

Date of mailing of the international search report

16 12 2002

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Smalt, R

## INTERNATIONAL SEARCH REPORT

Inte  
rial Application No  
PCT/US 01/23259

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE EMBL [Online] 19 October 1999 (1999-10-19) HASHIMOTO, K. ET AL.: "Mus musculus brain cDNA, clone:MNCb-4912, 5' end." Database accession no. AU079897 XP002223157 the whole document ---	8-14,48
Y	DAS, T. ET AL.: ".gamma.-Linolenic acid metabolism: Identification and characterization of .gamma.-linolenic acid elongation enzyme" INTERNATIONAL SYMPOSIUM ON .GAMMA.-LINOLENIC ACID, 2ND, SAN DIEGO, CA, UNITED STATES, 25 - 28 April 2000, XP008011237 In: .gamma.-Linolenic Acid: Recent Advances in Biotechnology and Clinical Applications (2001) Editors: Huang, Yung-Sheng; Ziboh, Vincent A. Publisher: AOCS Press, Champaign, Ill. the whole document	51,53, 63-72, 74-82
A	KENDRICK A ET AL: "LIPIDS OF SELECTED MOLDS GROWN FOR PRODUCTION OF N-3 AND N-6 POLYUNSATURATED FATTY ACIDS" LIPIDS, CHAMPAIGN, IL, US, vol. 27, no. 1, 1992, pages 15-20, XP002047887 ISSN: 0024-4201 the whole document ---	
X	SALEM N ET AL: "Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, US, vol. 93, January 1996 (1996-01), pages 49-54, XP002131822 ISSN: 0027-8424 the whole document	25,73
X	FR 2 648 347 A (SEDERMA SA) 21 December 1990 (1990-12-21) the whole document	25,73
X	EP 0 296 751 A (EFAMOL HOLDINGS) 28 December 1988 (1988-12-28) the whole document	25,73
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## INTERNATIONAL SEARCH REPORT

In — national Application No  
PCT/US 01/23259

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 07577 A (SCHERING BIOTECH CORP ;UNIV JOHNS HOPKINS (US)) 6 October 1988 (1988-10-06) the whole document ---	47,49, 50,52, 54,55
A	BOWLES R D ET AL: "Long-chain n-3 polyunsaturated fatty acid production by members of the marine protistan group the thraustochytrids: screening of isolates and optimisation of docosahexaenoic acid production" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 70, no. 1-3, 30 April 1999 (1999-04-30), pages 193-202, XP004173399 ISSN: 0168-1656 the whole document ---	
A	WO 98 46765 A (THURMOND JENNIFER ;CALGENE LLC (US); ABBOTT LAB (US); KNUTZON DEBO) 22 October 1998 (1998-10-22) example 1 ---	
P,X	WO 00 70945 A (KAROLINSKA INNOVATIONS AB ;ASADI ABULFAZL (SE); NEDERGAARD JAN (SE) 30 November 2000 (2000-11-30) see seq.ID's 5 and 6 ---	1-24, 26-34
E	WO 01 59128 A (ZAEHRINGER ULRICH ;BASF AG (DE); HEINZ ERNST (DE); LERCHL JENS (DE) 16 August 2001 (2001-08-16) the whole document -----	27,47, 49,50, 52,54, 55,73

**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/US 01/23259**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
  
The applicant timely paid all search fees for which he received an invitation to pay. However, due to the subsequent finding of a further non-unity within the second group of inventions, this international search report covers only the following: Inventions 1, 2.1, 3, 4: claims 1-34 and 47-82.
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-7, 15-24, 26-34 completely,  
and claims 8-14 partially

Elongase encoded by seq.ID.5, nucleic acids encoding it and homologs thereof, method for producing the enzyme, vector, host cell, transgenics, method for producing polyunsaturated fatty acids using the said elongase.

2. Claims: 25, 35-46, 73, 83-94 completely

Compositions comprising various kinds of unsaturated fatty acids and their nutritional, pharmaceutical, and cosmetic uses.

Subsequent finding of non-unity after payment of additional search fees; inventions:

- 2.1 Plant oil: claims 25 and 73.
- 2.2 Nutritional composition comprising DGLA: claims 35,36,39,83,84,87, all partially.
- 2.3 Nutritional composition comprising 20:4n-3: claims 35,36,39,83,84,87, all partially.
- 2.4 Nutritional composition comprising ADA: claims 35,36,38,39,83,84,86,87, all partially.
- 2.5 Nutritional composition comprising .omega.6-docosapentaenoic acid: claims 35-37,39,83-85,87, all partially.
- 2.6 Nutritional composition comprising STA: claims 35,36,39,83,84,87, all partially.
- 2.7 Nutritional composition comprising DGLA: claims 35,36,39,83,84,87, all partially.
- 2.8 Nutritional composition comprising AA: claims 35,37,39,83,85,87, all partially.
- 2.9 Nutritional composition comprising EPA: claims 35,37,39,83,85,87, all partially.
- 2.10 Nutritional composition comprising docosahexaenoic acid: claims 35,37,39,83,85,87, all partially.
- 2.11 Nutritional composition comprising .omega.3-docosapentaenoic acid: claims 35,38,39,83,86,87 partially.
- 2.12 Pharmaceutical composition comprising polyunsaturated fatty acids: claims 40,46,88,94.
- 2.13-2.24: Animal feed comprising unsaturated fatty acids, analogous to inventions 2.1-2.11 above, whereby claims 41-44 and 89-92 correspond to claims 35-39 and 83-87, respectively.
- 2.25: Cosmetic composition comprising polyunsaturated fatty acids: claims 45,93.

3. Claims: 47-49 completely, and claims 8-14 partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Elongase encoded by seq.ID.6, nucleic acids encoding it and homologs thereof, method for producing the enzyme, vector, host cell, transgenics, method for producing polyunsaturated fatty acids using the said elongase.

4. Claims: 50-72, 74-82 completely

Elongase encoded by seq.ID.7, nucleic acids encoding it and homologs thereof, and method for producing the enzyme.

Elongases with activity for unsaturated long chain fatty acids are known from e.g. WO0012720 and WO8807577. In particular, the mouse derived elongase encoded by the nucleic acid with seq.ID.5, is known from Tvrđik et al (Mai 2000) JCB 149(3):707-17. The fatty acids produced by the enzyme as claimed for the second invention identified above, and their use in e.g cosmetic and pharmaceutical, are all well-known in the art, as is evident from e.g. Salem et al (1996) PNAS 93:49-54, FR2648347, and EP296751.

In the light of this prior art, the first problem underlying the present application has been defined as the provision of further elongases and nucleic acids encoding them.

The solutions to this first problem lie in the provision of:

- seq.ID.5,
- seq.ID.6, and
- seq.ID.7, and their applications.

In the light of the prior art, the second problem underlying the invention has been defined as the provision of alternative uses for known fatty acids.

The solution to this problem lies in the compositions and uses and disclosed and claimed in invention 2, identified above.

In view of the fact that elongases with activity for unsaturated fatty acids are already known, due to the fact that the claimed fatty acids and their uses are also known, due to the essential difference between the two problems and their solutions, due to the essential difference in primary structures and putative functions of the elongases of the solutions to the first problem, and since no other special technical feature, common to these problems and their solutions could be distinguished, the ISA is of the opinion that there is no single inventive concept underlying the plurality of claimed inventions of the present application within the sense of Rule 13.1 PCT. Consequently there is a lack of unity and the different inventions, not belonging to a common inventive concept, are formulated above as the different subjects on the communication pursuant to Art. 17(3)(a) PCT.

The applicant has received the invitation pursuant to Rule 40.1 PCT and has paid additional search fees for (group of) inventions 2-4.

The International Searching Authority considers that for invention 2 a further lack of unity in view of the prior art as disclosed in Salem et al (1996) PNAS 93:49-54, FR2648347, and EP296751. All of the claimed unsaturated fatty acids are known, and many (if not all) of the uses

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

indicated are also known. An alternative method for producing these fatty acids does not render the compounds themselves or their uses new. These compounds and their uses are not linked by a single general inventive concept (Rule 13.1 PCT) or the same or corresponding special technical features (Rule 13.2 PCT)). Consequently, each use of each claimed compound constitutes a separate invention. The subdivisions have been indicated above under the header of invention 2.

Only the first invention in this group of inventions will be the subject of a search. No further invitation to pay further additional fees will be issued. This is because Article 17(3)(a) PCT stipulates that the ISA shall establish the International Search Report on those parts of the international application which relate to the invention first mentioned in the claims ('main invention') and for those parts which relate to inventions in respect of which the additional fees were paid. Neither the PCT nor the PCT guidelines provide a legal basis for further invitations to pay further additional search fees (W17/00, point 11 and W1/97, points 11-16).

This International Search Report in respect of former invention 2 has therefore been limited to the polyunsaturated plant oils of claims 25 and 73.

The applicant is advised that the EPO policy when acting as International Preliminary Examining Authority is normally not to carry out a preliminary examination on non-unitary matter which has not been searched (Rule 66.1(e) PCT). This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any International Preliminary Examination procedure.

**INTERNATIONAL SEARCH REPORT**

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Int'l Application No	PCT/US 01/23259
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**INTERNATIONAL SEARCH REPORT**

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Int'l	Int'l Application No
PCT	US 01/23259

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